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## CROPS : PLANT GROWTH : FERTILISERS.

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## STATISTICAL METHODS AND RESULTS.

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## THE SOIL.

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## SOIL CULTIVATION.

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## PHYSICAL PROPERTIES.

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31. "Further Note on the Capillary Forces in an Ideal Soil." R. A. FISHER. Jour. Agric. Sci. Vol. XVIII., pp. 406—410 ... 1928
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## CHEMICAL PROPERTIES.

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## SOIL ORGANISMS.

## BACTERIA.

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36. "The Rôle of the Young Lucerne Plant in Determining the Infection of the Root by the Nodule-forming Bacteria." H. G. THORNTON. Proc. Royal Society. B. Vol. 104, pp. 481—492 ... 1929
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## PROTOZOA.

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43. "Carbon Dioxide Production in Sands and Soils in the Presence and Absence of Amœbæ." D. W. CUTLER and L. M. CRUMP. Ann. App. Biol. Vol. XVI., pp. 472—482 1929

## FUNGI.

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## ALGÆ.

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## THE PLANT IN DISEASE: CONTROL OF DISEASE.

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53. "English-grown Pyrethrum as an Insecticide. I." J. C. F. FRYER, F. TATTERSFIELD and C. T. GIMINGHAM. *Ann. App. Biol.* Vol. XV., pp. 423—445 ... 1928
54. "Laboratory Experiments with Non-Arsenical Insecticides for Biting Insects." C. T. GIMINGHAM and F. TATTERSFIELD. *Ann. App. Biol.* Vol. XV., pp. 649—658 ... 1928
55. "Pyrethrin I. and II. Their Insecticidal Value and Estimation in Pyrethrum (*Chrysanthemum Cinerariæfolium*). I." F. TATTERSFIELD, R. P. HOBSON and C. T. GIMINGHAM. *Jour. Agric. Sci.* Vol. XIX., pp. 266—296 ... 1929
56. "Pyrethrin I. and II. Their Estimation in Pyrethrum (*Chrysanthemum Cinerariæfolium*). II." F. TATTERSFIELD and R. P. HOBSON. *Jour. Agric. Sci.* Vol. XIX. pp. 433—437. ... 1929

## FUNGUS PESTS AND THEIR CONTROL.

57. "The Toxicity of Certain Sulphur Compounds to *Synchytrium Endobioticum*, the Fungus Causing Wart Disease of Potatoes." W. A. ROACH and M. D. GLYNNE. *Ann. App. Biol.* Vol. XV., pp. 168—190 ... 1928
58. "Additional Hosts of *Synchytrium Endobioticum* (Schilb.) Perc." M. S. MARTIN. *Ann. App. Biol.* Vol. XVI., pp. 422—429 ... 1929
59. "Sulphur as a Soil Fungicide against the Potato Wart Disease Organism." W. A. ROACH. *Jour. Agric. Sci.* Vol. XX., pp. 74—96 ... 1930

## VIRUS DISEASES.

- |     |  |      |
|-----|--|------|
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## BACTERIAL DISEASE.

- |     |  |      |
|-----|--|------|
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| 64. | “Thionin and Orange G for the Differential Staining of Bacteria and Fungi in Plant Tissues.” R. H. STOUGHTON. Ann. App. Biol. Vol. XVII., pp. 161—164 ... ..             | 1930 |

# The Phosphate Requirement of Barley at Different Periods of Growth.

BY

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With Plate III and nine Figures in the Text.

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THE growing realization of the importance of phosphorus in plant nutrition has led to a good deal of attention being focused on the periods of the life-history in which the element is of the greatest value to the plant. The question of economy in phosphate utilization and supply has a very direct bearing on agricultural practice, and the experiment here to be considered has been undertaken with this in mind. On arable land phosphatic manures are generally applied before or at the time of sowing, to ensure that they are properly mixed with the soil, and the soundness of this procedure as regards plant nutrition receives further support from the results here presented.

## SCHEME OF EXPERIMENT.

With a view to testing the effect of alteration in the phosphate supply at different growth periods, experiments were made in which barley plants were grown for varying times in solutions containing phosphate, and were then transferred to other solutions minus phosphate. Vice versa, plants

grown without phosphate at first ultimately completed their growth in the presence of a full supply of phosphate, the successive transfers of five plants each being made in both cases at fortnightly intervals. This gave two series of plants grown respectively with and without phosphate for 0, 2, 4, 6, 8, 10, 12, 14, and 16 weeks, then receiving the reverse treatment. Simultaneously two series of five plants each, started with and without phosphate respectively, were changed into the reverse solution at the end of every fortnight, giving a double set which only had phosphate available for half-time, evenly distributed throughout the growth period. It was hoped that this might indicate whether the temporary presence or absence of phosphate was more critical at any one point in the life-history than at another. At the time of each transfer five similar plants from both original sets ( $\pm P$ ) were determined for dry matter and phosphate content, giving a measure of the position when each change of treatment occurred. The plants were grown singly in bottles of 600 c.c. capacity, and the solutions were changed weekly. The roots of the plants undergoing transfer were carefully washed to remove as much of the old solution as possible, to avoid carrying over more phosphate than could be avoided, the no phosphate roots being washed for the sake of uniformity.

The solutions used were as follows:

	With Phosphorus.	Without Phosphorus.
Potassium nitrate . . . . .	1 grm.	1 grm.
Potassium di-hydrogen phosphate . . . . .	0.3 "	—
Potassium mono-hydrogen phosphate . . . . .	0.27 "	—
Sodium chloride . . . . .	0.5 "	0.5 "
Magnesium sulphate . . . . .	0.5 "	0.5 "
Calcium sulphate . . . . .	0.5 "	0.5 "
Potassium chloride . . . . .	—	0.4 "
Ferric chloride . . . . .	0.04 "	0.04 "
Water . . . . .	to make up 1 litre (pH 6.2)	to make up 1 litre (pH corrected to 6.2 by the use of NaOH solution)

The complete nutrient solution supplied phosphorus at the rate of 116.5 parts per million, equivalent to 266.7 p.p.m.  $P_2O_5$ . Analyses made of average plants at fortnightly intervals showed a maximum uptake of 0.1646 grm.  $P_2O_5$  during two weeks of active growth with two changes of solution. This represents 0.0823 grm. per week, equivalent to 82.3 p.p.m.  $P_2O_5$  taken up from a solution originally containing 226.7 p.p.m. Thus it is clear that an ample reserve of phosphate was always available for use throughout the periods that phosphorus was supplied, and no question of deficiency effect due to inadequate rationing of phosphorus can therefore arise.

A pure strain of Goldthorpe barley was used, graded between 0.04 and 0.05 grm. weight, sown in sawdust April 1st, 1927, and put into nutritive solutions April 8th. The seed coats showed a tendency to develop mould, and as soon as the endosperm was absorbed they were removed and

no further trouble occurred from that source. Week by week the number of tillers on each plant was counted, each new tiller being labelled by number. Towards the end of the experiment many tillers died, and the progress of this decrease was also recorded.

The plants that bore ears were harvested as they became ripe, when all green colour had disappeared from grain and awns, cutting being spread over three weeks. Even at the end of that time a few of the tiny plants grown without phosphate were still green, but nothing would have been gained by keeping them longer. Most of the plants without ears were cut eight days after the beginning of harvest, in a succulent green condition, as they tended to die off in a decayed state if left too long.

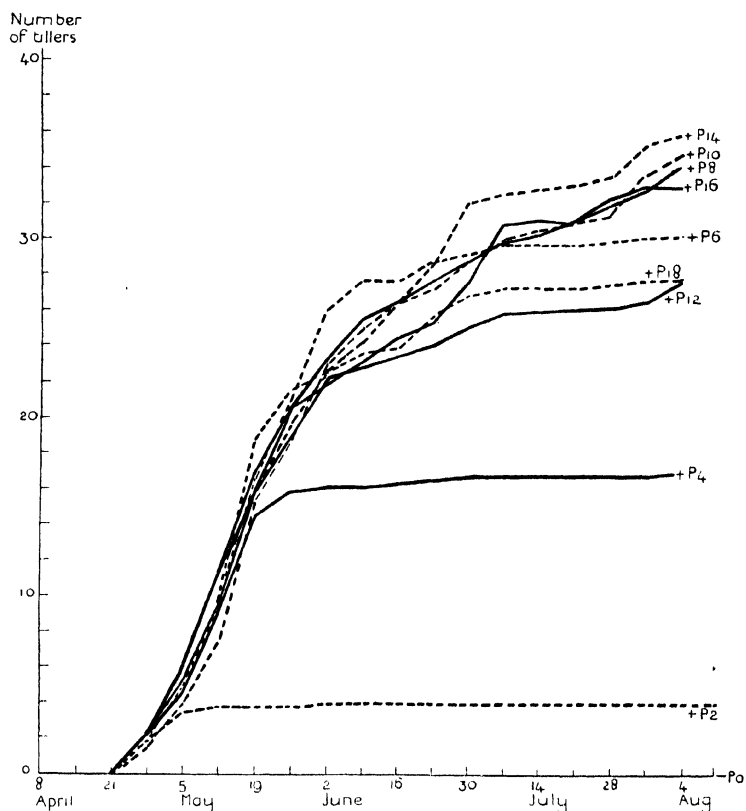
At the time of harvest each earing tiller was measured from the base to the middle of the ear, and the number of grains and sterile flowers from each ear was counted. Shoot, leaves, root, grain and sterile flowers were weighed separately, and to the leaves and shoots were added those which had died during growth and been kept till required. The dry weights only were recorded, and phosphate determinations were made on grains and sterile flowers together, shoot and leaves together, and on the roots. The latter presented difficulty, as it was impossible to be certain that all the phosphate from the nutritive solution was removed from the root in spite of most careful and prolonged washing in many changes of water when harvested. When the analytical figures became available, however, it was obvious that the washing had been more complete and successful than was hoped for. (Data given in Appendix A.)

#### TILLER PRODUCTION.

When phosphate was *present* at the outset tiller formation began from two to three weeks after the seedlings were put into solutions and proceeded at a rapid rate, an average of twenty tillers per plant appearing in the next four weeks, after which the rate began to slacken off. During the first period all the plants were very uniform in behaviour, as the total range of variation for groups of five plants did not exceed three tillers (Text-fig. 1, May 26th). During the later period the majority of new tillers were small and insignificant, and tended to die off without developing much. Individual plants varied greatly in this respect, causing considerable divergence in the tiller numbers for groups of five plants. This divergence set in weeks before any change of treatment occurred in those plants which grew with phosphate for twelve to sixteen weeks, and therefore was not connected in any way with the removal factor. Considering the curves for all treatments, it therefore seems probable that there is little or no significant difference in the total number of tillers produced, provided phosphorus is available during the first six weeks. If it is only present for four weeks, tiller formation continues for another fortnight as rapidly as where phosphorus is still

available, but then is suddenly checked for all time. If the removal occurs after a fortnight three or four tillers are produced during the next two weeks, and then formation ceases except in isolated cases.

When phosphate was *withheld* for the first fortnight, the number of tillers produced was slightly depressed for about two months, after which

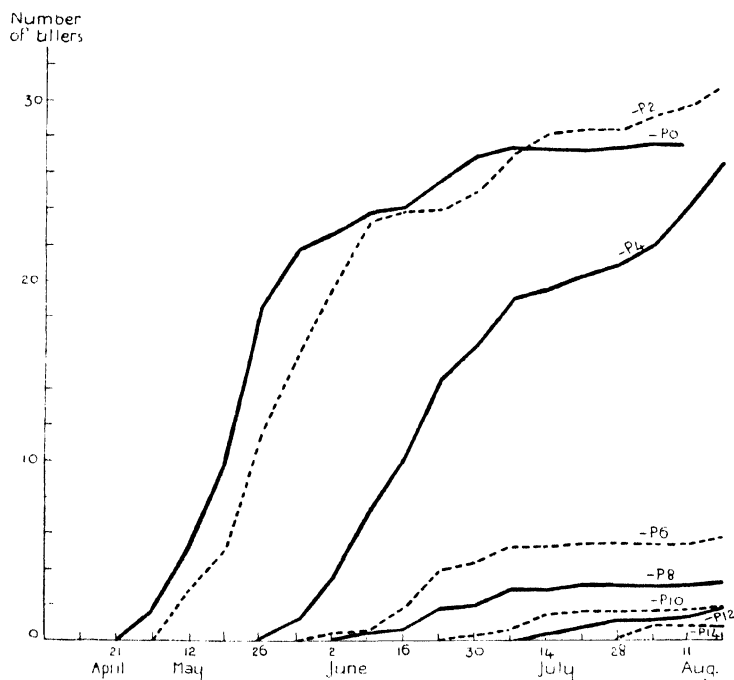


TEXT-FIG. 1. Graph showing total number of tillers formed in barley grown with phosphate at first, then without phosphate. (+ P<sub>2</sub>, &c., indicate number of weeks with phosphate.)

there was no significant difference from the plants which had received phosphate all through until towards harvest time, when the initially deprived plants were still forming new tillers (Text-fig. 2). After four weeks without phosphorus, tiller production proceeded at as rapid a rate as in the above case, but showed hardly any slackening at harvest, by which time about as many tillers were present as in the fully-fed plants. Longer periods of deprivation did not admit of so marked a recovery, a few tillers being formed when phosphorus was first supplied, after which the impetus seemed to cease. The energy of tiller production decreased as the period

without phosphate became longer, plants grown for sixteen weeks without phosphorus failing to show any response whatever, though some might still have been shown if the plants had been kept longer.

In both cases, whether phosphate was initially supplied or withheld, the critical period seemed to lie between the first four and six weeks, a definite change in the direction of the effect of the treatment occurring between



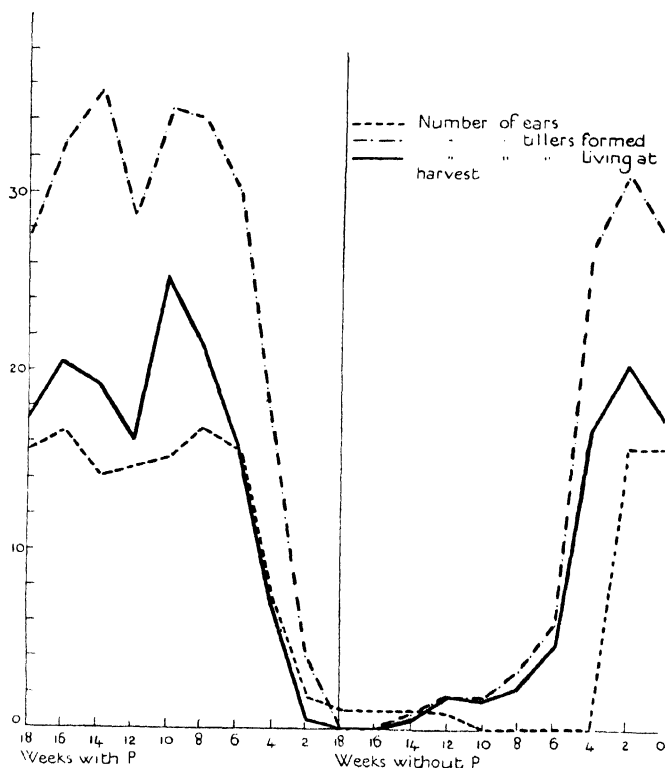
TEXT-FIG. 2. Graph showing total number of tillers formed in barley grown without phosphate at first, then with phosphate. (—P<sub>2</sub> &c, indicate number of weeks without phosphate.)

these dates. The considerable length of time that plants starved of phosphorus retain their power of responding by tiller production when phosphorus is given is worthy of note.

### EAR FORMATION.

Provided phosphate was available for the first six weeks, removal at any later date did not significantly affect the number of ears produced, which amounted, roughly speaking, to about one-half the total number of tillers formed (Text-fig. 3). With shorter periods in phosphate ear production declined rapidly, the same ratio to total tiller formation being maintained approximately. The majority of the ears were produced by the earliest tillers, the reduction in tiller numbers being mainly due to the death

of those formed later in life. In some cases, however, where phosphate was presented for a limited time at the beginning there was a tendency for one or more of the very first tillers to fail to produce ears. The reduction in the number of ears was accompanied by a reduction in size and quality, and those ears with only two weeks in phosphate tended to die off without emerging properly, before the grains had filled out. Despite variation in



TEXT-FIG. 3. Graph showing correlation between (a) total number of tillers formed; (b) number of living tillers at harvest; (c) number of ears produced in barley, as affected by varying phosphate treatment.

size, all the plants started with phosphate were of similar type, showing erect tillers and ears, though leaf production was more prolific with some treatments, as will be shown later (Pl. III, Fig. 1).

When phosphate was *omitted* during the early history a different course of events resulted. Omission for the first two weeks had no adverse effect, but omission for four weeks changed the character of the plant completely and entirely inhibited ear formation (Pl. III, Fig. 2). The number of tillers ultimately formed was much the same in both cases, but with four weeks omission of phosphate they were very sappy and flabby in nature, readily

attacked by mildew, and altogether unhealthy and abnormal in type. The large number of tillers and absence of ears gave the plants a very curious and characteristic appearance. As the time without phosphate increased to ten weeks the type of growth remained the same, but the number of tillers fell rapidly and the plants became very small. With twelve weeks and more without phosphate the growth type again changed, the succulence of the few tillers (if any) disappeared, and the thin feeble main shoot in most cases developed a small badly-formed ear, with very few grains, of which most were sterile. The need of phosphate very early in the life-history is clearly shown, and is evidently associated primarily with ear formation. Its absence at the critical moment abruptly checked this, but did not at the same time affect tiller production, thus accounting for the large bunchy plants produced after four weeks without phosphate. Longer periods of deprivation reduced tiller production until a point was reached when a characteristic 'no phosphate' type was fixed, in which the energy of the plant was directed to the formation of a travesty of an ear at the expense of the phosphorus stored in the seed, little or no attempt at tiller formation being made.

#### GRAIN FORMATION.

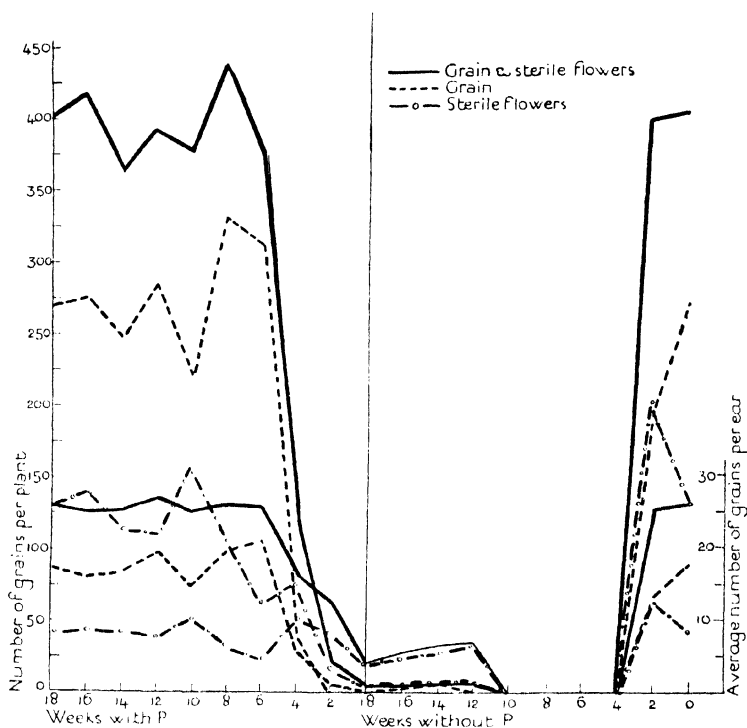
Provided phosphate was present for the first six weeks, little significant difference occurred in the total number of grains produced if it were withheld at any later date (Text-fig. 4). The largest proportion of grains to sterile flowers occurred with six weeks phosphate, the proportion being less with increased phosphate provision, though there was no regularity in the variation of the ratio with different periods of time. When phosphorus was only available for the first four weeks or less the total number of grains fell below that of the sterile flowers, until with entire absence of phosphorus no grains at all were produced.

The course of events for the average number of grains per ear was very similar to the above, showing that the decrease in the number of ears due to acute phosphate deficiency (Text-fig. 3) was paralleled by a similar reduction in grain formation per ear. The close similarity in the average number of grains per ear provided phosphate was given for six weeks or longer, was noteworthy, the maximum variation being two grains per ear.

The omission of phosphate at the outset was of far greater significance to grain formation than its removal at any time after initial supply. The absence of phosphorus for the first fortnight did not affect the total number of flowers produced, but it so decreased the grains and increased the sterile flowers that the proportions of the two classes were equal instead of being in the ratio of 2/1 as when phosphorus was supplied throughout. As already noticed, ear and grain formation were entirely inhibited with longer periods of phosphate omission up to twelve weeks, at which point feeble single ears

were produced with a larger proportion of sterile flowers to grains. The actual number of grains and sterile flowers per ear tended to decrease somewhat with lengthened periods of phosphate omission, being least where no phosphate was supplied at any time.

It is evident from these results that the critical period of need of phosphate for grain formation is at the beginning of development and not at the



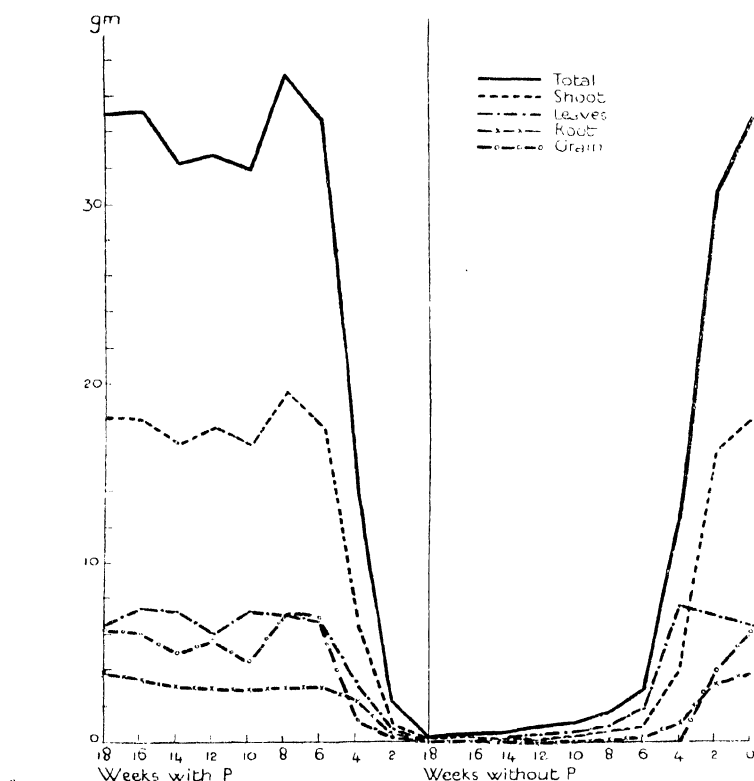
TEXT-FIG. 4. Graph showing effect of varying phosphate treatment upon (a) total number of grains and sterile flowers per plant; (b) average number of grains and sterile flowers per ear.

end, the necessity being most marked between the second and fourth weeks, and fully supplied by the sixth, under the particular environmental conditions of the experiment. Even the first fortnight is of significance, as provision for that period alone tripled the flower production per ear compared to that when no phosphorus was supplied (twelve to four), whereas omission for the same period influenced development and reduced the proportion of grains to sterile flowers (from two: one, to one: one).

#### DRY WEIGHT.

The dry weights of the whole plant, and of its component parts, were not significantly affected *provided phosphate was supplied* for the first six

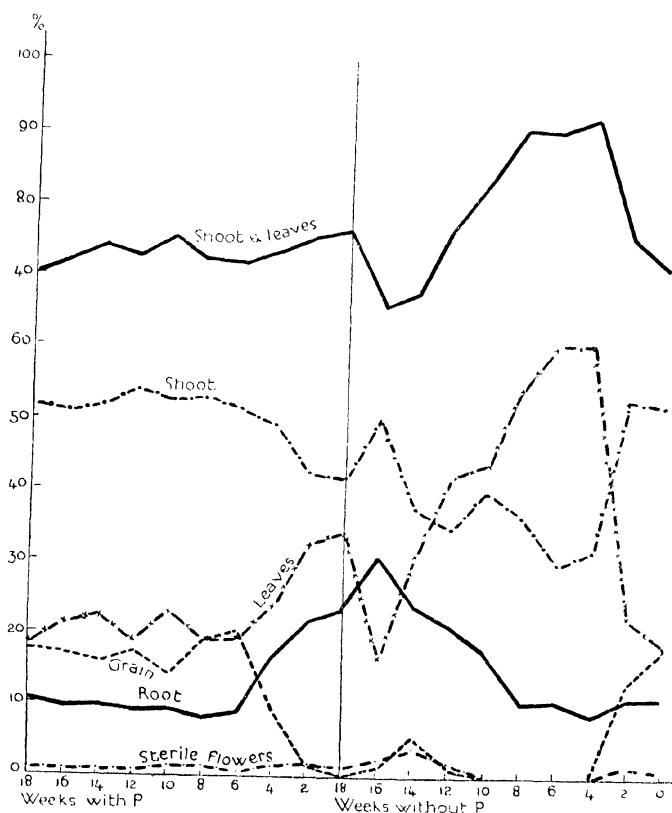
weeks or longer, unless the heavier weight of grain and shoot with the eight weeks' period should prove to be real after further experiments. Shorter treatment with phosphate caused a sharp depression of dry weight, specially marked in the shoot and grain, less evident in the leaves and root (Text-fig. 5). The proportion of each component to the total dry weight was unaffected down to the six weeks period, but the reactions varied with



TEXT-FIG. 5. Graph showing actual dry weight of barley and its component parts as affected by varying phosphate treatment.

shorter times of phosphate provision. The percentage of the total dry weight provided by the grain declined very rapidly under these conditions, the shoot showed a definite but less marked decrease, whereas the leaves and root increased steadily in proportion as the time of phosphate supply was reduced to nil (Text-fig. 6). This latter phenomenon is always very obvious when the plants are handled during growth. The proportion of stem and leaf together, however, is very little affected by the time of initial exposure to phosphate, and the actual variation in proportion lies between the root and grain in such cases of phosphate deficiency.

The omission of phosphate even for the first fortnight caused some decrease in the dry weight of all parts of the plant except the leaves, and deprivation for a month accentuated the fall, but still left the leaves unaffected (Text-fig. 5). The slight rise shown is unlikely to be significant. The marked decline of the various components continued with six weeks

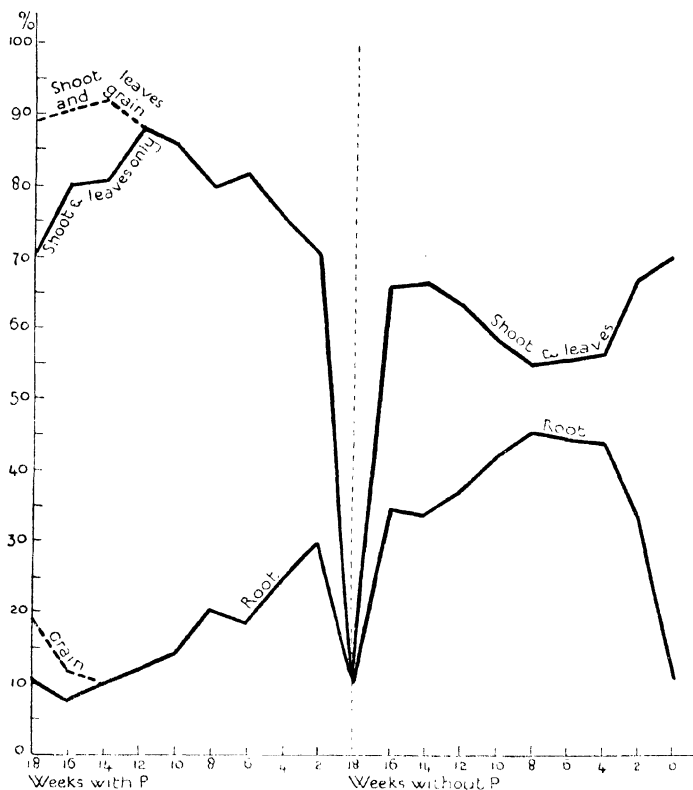


TEXT-FIG. 6. Graph showing effect of varying phosphate treatment on the percentage proportion contributed by the different parts of the barley plant to the total dry weight.

absence of phosphate, and at this time the leaves were adversely affected to the same degree. After this the dry weights continued to decrease slowly with longer periods without phosphate, the total weight being only 0.356 grm. in the entire absence of phosphate supply. A striking effect of the initial omission of phosphate is that the proportion of leaf is higher than that of stem for the intermediate periods (Text-fig. 6), the difference being very great with from four to eight weeks' omission and decreasing with longer periods, until with fourteen weeks' starvation the normal order reasserts itself and the stems outweigh the leaves. The proportion of root to total shoot

is little affected until after eight weeks' phosphate omission, when it rises rapidly and steadily with the increase of time of starvation.

Under normal conditions of nutrition the dry weight of the entire shoot (stem, leaves, and grain) increases in proportion, at the expense of the root, till a few weeks before harvest, in this case forming ultimately about 90 per cent. of the total (Text-fig. 7). This is also the case if phosphate is pro-



TEXT-FIG. 7. Graph showing percentage proportion contributed by different parts of the barley plant to total dry weight at different periods of growth.

vided for six weeks or longer, otherwise the root becomes proportionately heavier as the initial time of phosphate supply is lessened (Text-fig. 6). When phosphate is not available at the beginning, the ratio of shoot to root is throughout less than in the latter case. As the period of omission increases, the proportion of root rises rapidly at first, but after about eight weeks' omission, when the type of growth is changing (see p. 95) root development becomes less rapid than that of the shoot, the difference being the more marked the longer the period of initial phosphorus starvation. This variation again emphasizes the importance of the first few weeks in the

phosphate nutrition of the plant, the whole course of the life-history being influenced by the conditions at that stage far more than by changes at later dates.

#### PHOSPHATE UPTAKE.

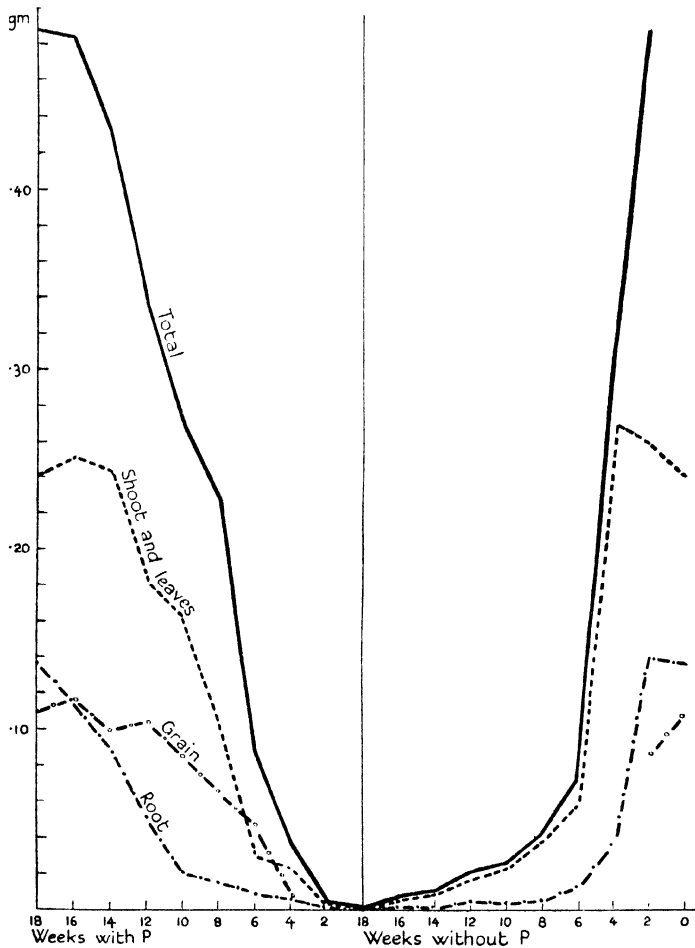
##### (a) *Phosphate present at beginning.*

Very little phosphorus was absorbed when phosphate was presented during the first fortnight only, but with longer periods of initial presentation the uptake increased rapidly, practically in direct proportion to the length of time phosphate was available in the solution (Text-fig. 8). A few weeks before harvest, however, the uptake at first slackened slightly, and then entirely ceased during the later stages of ripening of the grain, the presence of phosphate in the solution at this time being therefore redundant. Phosphate uptake under these conditions corresponds closely to that recently shown by Arrhenius (1) for barley grown in water cultures and analysed for phosphate at fortnightly intervals. It is very significant that sufficient phosphate was absorbed in the first six weeks to enable the plant to make its maximum amount of dry matter, the necessary percentage of  $P_2O_5$  amounting to 0.25 per cent. of the ultimate dry matter formed. The further supplies of phosphate absorbed during later periods had no influence on dry matter production, merely increasing the percentage of phosphate present. This is a further indication of the importance of phosphate supplies in the early history of the plant, the later supplies having little effect upon yield, though they improve the quality of the crop as far as nutritive value is concerned. The general trend of this result corresponds with that obtained by Pember (5), who states that during the first three weeks of growth in culture solutions his barley plants absorbed enough  $P_2O_5$  to produce plants maturing nearly the maximum amount of seed. Pember and McLean (6) also indicate the very small amount of phosphate necessary to produce a good yield, 0.2 per cent.  $P_2O_5$  in dry matter being adequate.

##### *Relation between Dry Weight and Phosphate Uptake.*

+ P Weeks.	Dry Weight Total.	$P_2O_5$ Total.
	gram.	gram.
0	0.36	0.0006
2	2.39	0.0039
4	14.08	0.0361
6	34.62	0.0878
8	37.16	0.2275
10	31.80	0.2700
12	32.62	0.3373
14	32.29	0.4321
16	35.16	0.4825
18	35.02	0.4871

The distribution of phosphate to the different parts of the plant varied considerably at different periods. In the *shoot* (including leaves) the percentage of  $P_2O_5$  was constant for periods of phosphate presentation up to

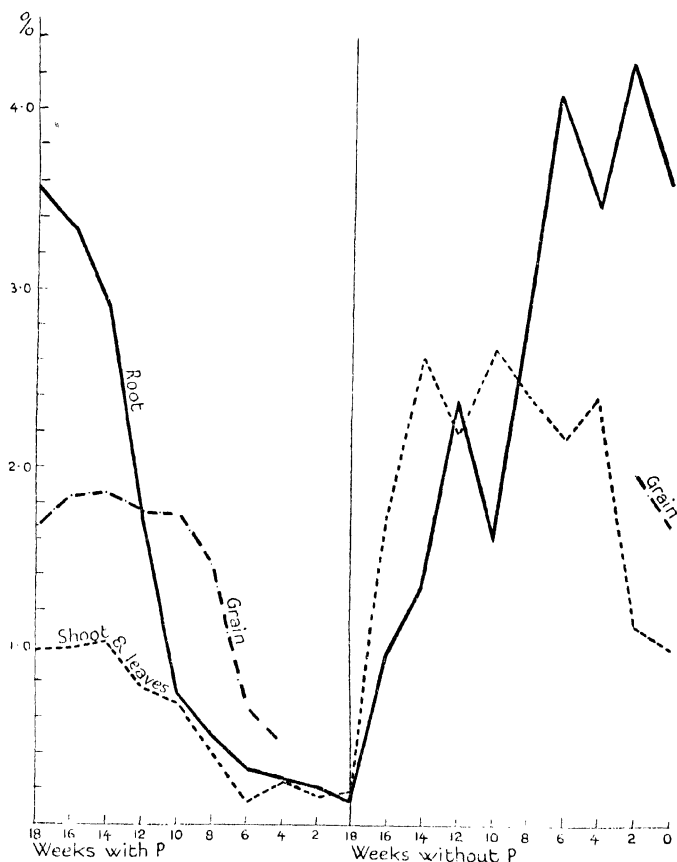


TEXT-FIG. 8. Graph showing actual phosphoric acid present in barley plants, as affected by varying phosphate treatment.

six weeks, after this the percentage increased steadily until about a month before harvest, and then again remained constant (Text-fig. 9). This variation in percentage corresponds with a relatively slow  $P_2O_5$  reception for the first six weeks presentation, followed by a rapid increase in quantity, until at a month before harvest the supply was abruptly cut off.

In the *grain* the percentage of phosphate increased rapidly until the initial time of presentation reached ten weeks, but with longer periods of

availability the percentage remained fairly steady. The greater part of the phosphate had passed into the grain after the first twelve weeks, the addition during the last six weeks being small. This compares well with the result obtained in barley grown under field conditions (2). In unmanured



TEXT-FIG. 9. Graph showing percentage of  $P_2O_5$  in dry matter of barley as affected by varying phosphate treatment.

soil the phosphate uptake of the whole plant ceased five weeks before harvest, and migration into the grain ceased about ten days later, whereas in manured soil the uptake continued rather longer, but ceased in the same way some time before harvest.

The phosphate uptake of the *root* was distinctly different from that of the rest of the plant. The  $P_2O_5$  percentage remained low at first, rising slowly up to ten weeks' initial presentation of phosphate, then proceeded much more rapidly to fourteen weeks' initial phosphate, after which the rate

slackened again. With more than twelve weeks' initial phosphate the percentage of  $P_2O_5$  in the root was considerably greater than in either the shoot or grain. The actual amount of phosphate in the roots was very small till the change with the ten weeks' set, but then rose rapidly and steadily until the end. It seems unlikely that the increase is due to extra phosphate remaining adherent to the surface of the root and not removed by washing, as during the time (ranging up to eight weeks) that the plants were grown

Total  $P_2O_5$  Present in Barley.

A. Plants transferred to solutions without phosphate at periods indicated.

B. Plants harvested at periods indicated.

After Weeks with Phosphate.	Shoot.		Root.		Grain.		Total.	
	A.	B.	A.	B.	A.	B.	A.	B.
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
2	0.0029	— ?	0.0010	— ?	—	—	0.0039	0.0014
4	0.0236	0.0051	0.0058	0.0074	0.0068	—	0.0361	0.0125
6	0.0317	0.0431	0.0095	0.0213	0.0467	—	0.0878	0.0644
8	0.1041	0.1154	0.0152	0.0601	0.1082	—	0.2275	0.1755
10	0.1626	0.2017	0.0218	0.0473	0.0855	—	0.2700	0.2490
12	0.1823	0.2952	0.0515	0.1184	0.1035	—	0.3373	0.4136
14	0.2439	0.3445	0.0907	0.1449	0.0976	0.0453	0.4321	0.5347
16	0.2510	0.3306	0.1154	0.1138	0.1161	0.0413	0.4825	0.4857
18	0.2409	0.2409	0.1367	0.1367	0.1095	0.1095	0.4871	0.4871

in solutions without phosphate, after their initial periods with phosphate, it may be expected that any adherent phosphate would be absorbed or washed off into the solutions, which were changed weekly. The indications are, therefore, that phosphate absorption from the solution continued to a very late date, only ceasing shortly before harvest. Earlier than this, however, migration into the shoot slackened and finally ceased, the excess phosphate remaining perforce in the root, instead of being distributed throughout the plant, as occurred earlier in the life-history. The question arises as to whether loss of phosphate from the plants took place during the periods during which they were growing in no phosphate solution. A comparison of the calculated amounts of  $P_2O_5$  present in these plants with others harvested at each fortnightly interval provides no definite evidence that this was the case, possibly until the plants were approaching maturity. For each of the earlier periods (2, 4, 6, 8, 10 weeks' initial phosphate) actually less  $P_2O_5$  was present in the plants sampled than in those grown on without phosphate, the difference obviously being covered by the variation in individual plants and the experimental error. For the next two or three periods the position was reversed, and it is possible that here some actual loss was incurred by the natural dying off of parts of the root at this stage of growth. The  $P_2O_5$  content of the root at the actual time of change was in general higher than at harvest time after growing in no phosphate solution, probably on account of phosphorus being transferred from the roots to

other parts when starvation occurred. This did not occur in the last two or three weeks when, as indicated before, migration within the plant has apparently ceased. The grain is obviously a first charge on the plant in the matter of phosphate supply, as the full complement was passed into the grain even when the plant received phosphate for the first eight weeks only. This stability was attained at the expense of the shoot, whose phosphate content at harvest was consistently lower than that of the samples taken at time of transfer to no phosphate solutions.

(b) *Phosphate absent at beginning.*

Phosphate deficiency in the early stages of growth had a drastic influence upon the uptake of phosphate, paralleled closely by its effect upon the dry weight. An extremely rapid drop occurred when phosphate was withheld from two to six weeks at the beginning, after which a more gradual decrease set in with lengthening periods of phosphate privation (Text-fig. 8). There was nothing here to parallel the high dry matter production with small phosphate uptake as occurred in the reverse series (see (a)).

With regard to the distribution throughout the plant, the bulk of the  $P_2O_5$  throughout was in the shoot. The first rapid drop in phosphate content in the root occurred after four weeks' initial starvation, a fortnight less than was needed to induce a similar drop in the shoot, indicating that the shoot is somewhat more resistant than the root in this respect. The percentages of phosphate in the shoot and root followed markedly different courses. In the shoot a very heavy percentage was present after periods of starvation ranging from four to fourteen weeks, suggesting a considerable intake when phosphate was eventually supplied, without, however, sufficient vitality or response on the part of the plant to enable it to utilize this phosphate in the production of additional dry matter (Text-fig. 9). In the root, on the contrary, the percentage of phosphate remained at a level much above that in the shoot, approximating to that of a normally fed plant until after six weeks' initial phosphorus starvation, and then suddenly and rapidly decreased more or less in proportion to the increase in the time phosphate was withheld, falling below the percentage in the shoot at this stage.

Pember's results are in striking contrast to these, as he found that after serious semi-starvation of phosphorus during the early stages barley was able to utilize abundant later supplies satisfactorily, storing much of it in the seed. The crux of the discrepancy would appear to lie in the different effect of *total* and *semi*-starvation in the early periods, but comparative experiments on this point are still wanting.

#### DISCUSSION.

Under the conditions of the experiment, in which phosphate was either entirely withheld or supplied in ample quantity for certain periods, it is

evident that it is during the early stages of growth that the presence or absence of phosphate is of most vital importance. When a sufficiency of phosphate was supplied for the first few weeks tillering, ear and grain formation and the production of dry matter proceeded as well and to the same degree as though phosphate had been present throughout. The uptake of phosphorus, however, did not follow the same course, but continued steadily until shortly before harvest, so that the percentage of phosphate present in the dry matter formed was correlated with the length of time that phosphate had been available in the solution. Similar conclusions were reached by Hoagland (4), who indicated that the yield is largely a condition of the supply of nutrients during the first few weeks of growth, and is but little affected by the later uptake. On the other hand, the absence of phosphate from the nutrient solution, just at the time tillering was due to begin, inhibited the formation of ears without reducing tiller production until the phosphate had been withheld for a rather longer initial period. This finding as to the vital necessity of phosphate in the early stages of development appears to run directly contrary to the conclusion of Pember and McLean (6), who found that barley could utilize a deficient supply of phosphate equally well whether it was supplied early, in the middle period, or late in the life of the plants. The explanation of this discrepancy may lie in the fact that well water was used throughout the American experiments, providing a certain amount of phosphate in every case over and above that used experimentally. It may be suggested that the actual requirement of the plant for phosphorus in the earliest days is exceedingly small, and that even a minimal amount supplied at this time ensures the proper laying down of the ears, which can then develop later, when more plentiful supplies of phosphate become available. In the total initial absence of phosphate for short periods the energy of the plant appears to be directed towards abundant tiller production at the expense of the ears, a tendency that would seem to be counteracted by the presence of small quantities of phosphate at this time. A further point to be considered is that Pember and McLean removed all tillers as they appeared, and only allowed the main shoot to develop. Consequently, any small supply of phosphate was concentrated in its sphere of action, and may have induced ear formation in cases in which it would have been insufficient if tillers had been allowed to develop. This is further exemplified by the Rothamsted plants, grown entirely without phosphate, which produced no tillers but developed one feeble ear plant, presumably at the expense of the phosphate contained in the seed.

The presence or absence of phosphorus from the nutrient solution at the time tillering actually begins has a definite effect upon the later development. Plants that were grown with and without phosphate for alternate fortnights throughout their life-history showed differences in development according to whether phosphate were present or absent during the second

fortnightly period, in which tillers began to appear. With phosphate *present* at this time, the plants were distinctly ahead throughout growth, the ears emerged some days earlier, and the weight of the grain and the total dry weight were greater than when phosphate was *absent* at the critical moment. In the latter case, the ears did not all emerge completely even when ripe, giving the plants a characteristic appearance of poor growth (Pl. III, Fig. 3). In both sets the dry weights of the grain, shoot, and the whole plant were considerably lower than when phosphate was supplied for the first six weeks and then withheld—i.e. over the whole period of eighteen weeks better growth was obtained with one-third the total possible supply of phosphate provided consecutively during the first six weeks than from one-half the supply provided intermittently throughout growth. This, again, may emphasize the importance of the early weeks, as in the alternating series less phosphate was available during these weeks than in the case of the others, although their total supply was greater.

Dry Weight.	+ P first 2 weeks then - P, + P alternately.	- P first 2 weeks then + P, - P alternately.
	gram.	gram.
Shoot	13.58	15.88
Leaves	8.03	7.00
Root	3.18	2.60
Grain	1.11	3.18
Sterile flowers	0.50	0.60
Total	26.40	29.26

Compare + P for 6 weeks, then - P.  
Dry weight 34.62 gram.

There would appear to be a certain limit of possible growth which is reached if phosphate is available for a certain consecutive period at the beginning of growth, but which is not attained if it is entirely withheld even for the first fortnight only, nor if an intermittent supply of phosphate is continued throughout growth.

Gericke (3), working with wheat, obtained the maximum dry weight when the plants were grown in complete nutrient solutions for four weeks and were then transferred to solutions devoid of phosphorus. Decrease occurred in the ultimate dry weight as the initial period with phosphate was lengthened, the plants receiving phosphate throughout being amongst those with the lowest dry weight. Barley, under Rothamsted conditions of growth, did not show a sharp fall to correspond with this, though the plants transferred after eight weeks with phosphate (corresponding in stage of development to the wheat at four weeks' old) were somewhat heavier than those transferred later. The difference, however, is relatively small when the error of experiment is taken into account. In the following table the two plants are compared, the first transfer being made at corresponding stages of development.

Wheat. With P. for	Barley.	Wheat. (Gericke.)	Barley. (Brenchley.)
Weeks.	Weeks.	gram.	gram.
4	8	51.2	37.2
6	10	41.7	31.8
8	12	40.7	32.6
10	14	31.4	32.3
Throughout	Throughout	32.0	35.0

The more closely the work of various investigators is studied, the stronger grows the impression that the requirement of cereals for phosphate at different growth periods is by no means fixed or definite, but is influenced by various factors, such as environmental conditions (season, spacing of plants, time of sowing, &c.), inter-action of other nutrients, and the degree of completeness of phosphate starvation induced at different periods of growth. Nevertheless, whatever the condition, the indications are that the most critical period for phosphate nutrition is during the early stages of growth, and that later provision of phosphate tends to affect the composition of the crop without having a corresponding effect upon yield.

#### SUMMARY.

1. Experiments have been made in water cultures to test the effect of depriving barley plants of phosphorus after varying initial periods during which it had been supplied, and, contrariwise, of supplying phosphorus after initial periods of deprivation.

2. The provision of phosphate for the first six weeks or longer permitted normal growth to be made, as was shown by the number of tillers, ears, and grains produced, the average number of grains per ear, and the dry weights. With shorter initial periods of phosphate supply growth was seriously depressed in all these respects.

3. If phosphate was withheld for the first four weeks, tiller production was not affected, but no ears were produced. With longer periods of initial deprivation growth was steadily depressed in all respects, and the type of growth gradually changed from a bushy succulent character to a thin, lanky, untillered plant bearing the travesty of an ear.

4. The amount of phosphate absorbed by the plant increased steadily in more or less direct proportion to the length of time phosphate was given at the beginning of growth, but sufficient was taken up in the first six weeks to enable the plant to make its maximum dry weight. The percentage of phosphate in dry matter rapidly increased from this time onwards.

5. The absence of phosphate supply up to the first six weeks of growth caused a extremely rapid drop in the amount of phosphate ultimately taken up by the plant, after which a more gradual decrease occurred with lengthening periods of phosphate deprivation.

6. The significance of the above results is discussed in relation to the

work of other investigators, and the probable importance of the presence or absence of phosphorus at the time tillering begins is indicated by reference to further experiments in which phosphate was supplied and withheld for alternate fortnights during growth.

My sincere thanks are due to Miss Katherine Warington, M.Sc., Miss Lizzie Kingham (both of Rothamsted), and Miss F. L. Stephens, B.Sc. (of Manchester) for assistance in the tedious work of marking and counting tillers; to Miss Signe Heinze, of Stockholm, for aid in translation; and to the Chemical Department of Rothamsted for the series of phosphate determinations, without which the paper would have lost much of its point.

*Data of Barley Plants grown with or without Phosphate for the first part of their Life history.*

(Averages of five replicates.)

Weeks with or without Phosphorus.	No. of Ears.	Dry Weight.				Percentage P <sub>2</sub> O <sub>6</sub> in Dry.				Actual P <sub>2</sub> O <sub>6</sub> .			
		Sterile Flowers.		Leaves.		Shoot + Leaves.		Grain + Sterile flowers		Shoot + Leaves.		Grain + Sterile flowers	
		grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.
+ P 18													
(All time)	15.6	6.233	0.365	6.460	3.840	35.020	1.660	0.98	3.560	0.1095	0.2409	0.1367	0.4871
+ P 16	17.0	5.997	0.347	7.438	3.466	35.162	1.830	0.99	3.330	0.1161	0.2510	0.1154	0.4825
+ P 14	14.2	4.968	0.277	7.258	3.138	32.291	1.860	1.02	2.890	0.0976	0.2439	0.0997	0.4322
+ P 12	14.6	5.023	0.291	6.126	3.032	32.618	1.750	0.77	1.700	0.1035	0.1823	0.0515	0.3373
+ P 10	15.4	4.517	0.366	6.656	2.976	31.801	1.740	0.68	0.732	0.0855	0.1626	0.0218	0.2609
+ P 8	15.4	6.977	0.433	7.126	3.668	37.162	1.460	0.39	0.494	0.1082	0.1041	0.0152	0.2275
+ P 6	16.6	6.948	0.178	6.672	3.132	34.624	0.655	0.13	0.303	0.0467	0.0317	0.0035	0.0879
+ P 4	7.4	1.270	0.213	3.360	2.344	14.083	0.456	0.23	0.247	0.0068	0.0236	0.0058	0.0362
+ P 2	1.6	0.038	0.039	0.770	0.523	2.389	—	0.16	0.197	—	0.0029	0.0010	0.0039
+ P 0													
= - P 18	1.0	—	0.004	0.121	0.083	0.356	—	0.20	0.137	—	0.0005	0.0001	0.0006
- P 16	1.0	0.006	0.011	0.075	0.138	0.455	—	1.74	0.940	—	0.0054	0.0013	0.0067
- P 14	1.0	0.024	0.017	0.131	0.104	0.440	—	2.60	1.320	—	0.0087	0.0014	0.0101
- P 12	0.8	0.013	0.016	0.381	0.193	0.920	—	2.18	2.360	—	0.0159	0.0045	0.0101
- P 10	—	—	—	0.464	0.182	1.068	—	2.64	1.580	—	0.0234	0.0029	0.0263
- P 8	—	—	—	1.54	0.176	1.716	—	2.39	2.730	—	0.0368	0.0048	0.0416
- P 6	—	—	—	0.886	0.313	3.007	—	2.15	4.050	—	0.0579	0.0127	0.0706
- P 4	—	—	—	3.910	1.666	12.438	—	2.37	3.430	—	0.2694	0.0366	0.3660
- P 2	15.6	3.975	0.496	6.998	3.282	30.961	1.960	1.12	4.240	0.0877	0.2599	0.1392	0.4868
- P 0	15.6	6.233	0.365	6.460	3.840	35.020	1.660	0.98	3.560	0.1095	0.2400	0.1367	0.4871

# LITERATURE CITED.

1. ARRHENIUS, O. : Forsök rörande kali- och fosforsyrenäringens betydelse för våra kulturväxter, 1927.
2. BRENCHEY, W. E. : Development of the Grain of Barley, *Ann. Bot.*, xxvi, p. 916, 1912.
3. GERICKE, W. F. : Salt Requirements of Wheat at Different Growth Phases. *Bot. Gaz.*, lxxx, pp. 410-25, 1925.
4. HOAGLAND, D. R. : Relation of the Concentration and Reaction of the Nutrient Medium to the Growth and Absorption of the Plant. *Journ. Agric. Res.*, xviii, pp. 73-117, 1919.
5. PEMBER, F. R. : Studies by Means of both Pot and Solution Culture of the Phosphorus and Potassium Requirements of the Barley Plant during its Different Periods of Growth. *Bull.* 169, *Agric. Expt. Stat.*, Rhode Island State College, p. 50, 1917.
6.                   and McLEAN, F. T. : Economical Use of Nitrogen, Phosphorus, and Potassium by Barley, Oats, and Wheat in Solution Cultures. *Bull.* 199, *ibid.*, pp. 1-53.

# EXPLANATION OF PLATE III.

Illustrating Dr. W. E. Brenchley's paper on Phosphate Requirements of Barley.

Fig. 1. Barley plants which received phosphate at first, but were deprived of it after varying periods of growth.

Upper line of figures +P indicates initial time with P.  
Lower „ „ „ -P „ „ later time without P.

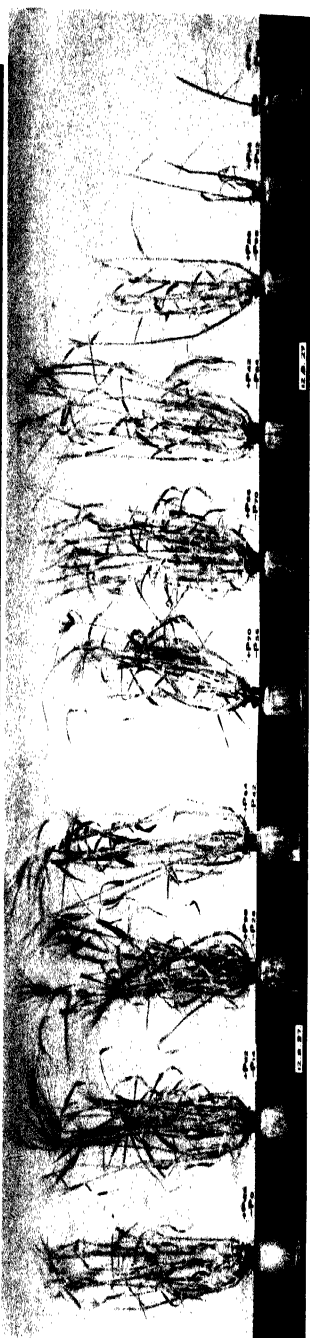
Fig. 2. Barley plants grown without phosphate at first, but which were supplied with it after varying periods of growth.

Upper line of figures -P indicates initial time without P.  
Lower „ „ „ +P „ „ later time with P.

Fig. 3. Barley grown with and without phosphate for alternate fortnights throughout growth.

Left-hand pair—without P for first fortnight.  
Right-hand „ „ with P for first fortnight.





BRENCHELY — PHOSPHATE REQUIREMENT OF BARLEY.

Rev. J. J. J. J.



## THE EFFECT OF FRESH STRAW ON THE GROWTH OF CERTAIN LEGUMES.

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(With Two Text-figures.)

IN field trials made at Rothamsted during 1917–1918<sup>(1)</sup> to test the comparative value of farmyard manure and of artificials as fertilisers for clover, the former gave remarkably better results. Its effect not only surpassed that produced by any mixture of artificials tested, in the year of application, but was evident even when applied three years before the clover was sown. In comparing the action of farmyard manure and of artificials, the effect of the former on the physical condition of such a heavy soil as that at Rothamsted must always be considered. In these trials, however, the superiority of farmyard manure was so much more marked with clover than with cereal and root crops on the same soil, that some further explanation seems to be required. Since clover is a legume, manures may have both a direct action on the plant, and may also affect its growth indirectly, by influencing the formation or activity of the nodules upon its roots. The purpose of the work here described was to determine whether farmyard manure stimulated nodule development on legumes and, if so, to what constituent of the manure this stimulation was due.

In order to separate the direct effect on the plant from any indirect influence through the nodules, farmyard manure and its constituents were first tested both on nodule free and on inoculated plants. Preliminary trials showed that, owing to the widespread occurrence of nodule organisms in soil, none of the common British legume crop plants could be grown without nodules, unless an unnatural medium such as sterilised soil or sand were employed. It was found, however, that the soy bean (*Glycine hispida*) does not produce nodules in Rothamsted soil unless infected with a culture of the appropriate variety of the nodule bacteria. This plant was therefore used in the first two experiments here described.

In the first experiment, pots of soil and sand mixture were prepared

## 564 *Effect of Fresh Straw on Growth of Certain Legumes*

and manured as shown in Table I<sup>1</sup>. There were ten pots of each treatment, five sown with seed sterilised by the method of Hutchinson and Miller(2) and five with sterilised seed inoculated with a suspension of the soy bean nodule organism. Three seedlings were allowed to grow in each pot and, after 13 weeks' growth in a glasshouse, the dry weights of tops and roots were obtained and the nodules counted, the soil being washed from the roots with a fine stream of water from a hose. The results of the experiments are shown in Table I.

Table I. *Pot experiment with soy beans.*

Series	Manuring per pot	Dry weight tops in gm. Means of 5 pots and standard errors	Dry weight roots (gm.)	Total nodules and standard errors	Nodules per gm. of root
Not inoculated:					
1	1 lb. farmyard manure	4.6 $\pm$ 0.92	1.67	—	—
2	$\frac{1}{2}$ lb. farmyard manure	5.4 $\pm$ 0.81	2.02	—	—
3	Extract from $\frac{1}{2}$ lb. farm- yard manure	8.1 $\pm$ 0.66	2.87	—	—
4	No manure	2.5 $\pm$ 0.17	1.95	—	—
5	$\frac{1}{2}$ lb. fresh chaff	1.5 $\pm$ 0.12	1.98	—	—
6	$\frac{1}{2}$ lb. rotted straw	3.0 $\pm$ 0.17	1.27	—	—
Inoculated:					
7	1 lb. farmyard manure	7.6 $\pm$ 0.63	1.51	146.2 $\pm$ 4.2	96.8
8	$\frac{1}{2}$ lb. farmyard manure	6.24 $\pm$ 0.61	1.75	153.2 $\pm$ 3.3	87.5
9	Extract from $\frac{1}{2}$ lb. farm- yard manure	8.56 $\pm$ 0.84	2.18	94.0 $\pm$ 2.5	43.2
10	No manure	2.3 $\pm$ 0.24	1.41	63.2 $\pm$ 3.0	44.8
11	$\frac{1}{2}$ lb. fresh chaff	2.0 $\pm$ 0.18	1.11	105.0 $\pm$ 4.0	94.6
12	$\frac{1}{2}$ lb. rotted straw	2.9 $\pm$ 0.41	1.28	65.0 $\pm$ 3.2	50.8

The manure and its extract have produced a considerable increase in the growth of the tops. This is mainly due to a direct action on the plant since it occurs in the absence of nodules. In the uninoculated series fresh straw has reduced the growth of the top as compared with the unmanured set and the farmyard manure which contained a lot of unrotted straw has produced less increase than the manure extract. The presence of nodules has not produced any significant increase in yield save in these same three sets (7, 8 and 11) that contained fresh straw. The yield of sets 7 and 8 containing farmyard manure are not significantly lower than that of set 9 containing manure extract, while there is no significant difference

<sup>1</sup> Each pot contained 23 lb. of a mixture of two parts soil and one part sand. The manure extract was prepared by soaking manure in water at the rate of 3 litres to 8 oz. Each plant was given the extract from 8 oz. manure during its growth period. The manure contained 0.92 per cent. nitrogen while the extract contained 0.43 per cent. nitrogen. Series 6 and 12 were given straw rotted by the "Adco" process.

in yield between the set containing chaff (11) and the unmanured<sup>1</sup>. The inoculation has thus prevented the straw from lowering the yield. This suggests that its depressing action in the uninoculated set was the result of nitrogen starvation due to the well-known effect of straw in rendering nitrates unavailable by encouraging their assimilation by micro-organisms. Where the nodule organism rendered the plant independent of external nitrogen compounds, this harmful action did not occur. In the inoculated sets, the manurial treatment has greatly affected the number of nodules produced. When allowance is made for unequal root development by taking the nodule numbers per gram of root, the infection has been increased only by chaff and by the manure containing straw. In the case of sets 7, 8 and 9, however, greater root development induced by manure and its extract caused the appearance of more nodules owing to the greater surface exposed to infection. The following experiment was made to see whether the increased nodule formation induced by unrotted straw could not be similarly enhanced by the

Table II. *Pot experiment with soy beans.*

Set	Manuring per pot	Dry weights of top and standard errors (gm.)	Dry weights of roots and standard errors (gm.)	Nitrogen in tops		Nodule nos. and standard errors	Nodules per gm. root
				%	Total content (gm.)		
Not inoculated:							
1	No manure	21.2 ± 2.5	---	1.56	0.33	—	—
2	4 oz. chaff	19.1 ± 1.4	---	1.99	0.38	—	—
3	3.3 gm. K <sub>2</sub> HPO <sub>4</sub>	28.8 ± 2.2	---	1.42	0.41	—	—
4	3.3 gm. K <sub>2</sub> HPO <sub>4</sub> + 4 oz. chaff	24.6 ± 2.2	—	1.37	0.34	—	—
Inoculated:							
5	No manure	23.1 ± 2.6	3.1 ± 0.3	3.16	0.73	121.8 ± 14.0	39.3
6	4 oz. chaff	26.7 ± 2.5	4.5 ± 0.5	3.61	0.96	179.8 ± 9.9	39.9
7	3.3 gm. K <sub>2</sub> HPO <sub>4</sub>	31.8 ± 3.5	6.4 ± 0.2	2.41	0.77	106.4 ± 4.8	16.6
8	3.3 gm. K <sub>2</sub> HPO <sub>4</sub> + 4 oz. chaff	33.0 ± 1.7	6.9 ± 0.13	2.96	0.98	316.8 ± 41.7	45.9

addition of soluble potassium phosphate to replace the potash and phosphate supplied in the manure. Soy beans were grown in pots, containing soil and sand mixture, which were given the manuring shown in Table II. The salts in sets 3, 4, 7 and 8 were applied as solutions during the growth

<sup>1</sup> In testing the significance of these differences, the *t* test was used (see Fisher<sup>(3)</sup>). In comparing the mean yield of tops in sets 8 and 9,  $t=2.2$ , giving a value of *P* between 0.1 and 0.05. In a similar comparison between sets 10 and 11,  $t=1.05$ , the value of *P* lying between 0.4 and 0.3.

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of the plants. Ten pots of each treatment were prepared, five sown with inoculated and five with sterilised seed. After 12 weeks' growth in a glass-house, the nodules were counted, dry weights taken and nitrogen estimations made of the tops. The results are shown in Table II.

The root development has been increased in the pots given potassium phosphate and in set 8 the further addition of chaff caused a large increase in nodule numbers. The presence of nodules has increased the nitrogen content in all inoculated sets but has caused a significant increase in the top growth only in sets 6 and 8 where chaff was present<sup>1</sup>. The chaff has enabled the plant to make some use of the nitrogen fixed by the bacteria, to increase its growth. The action of chaff in increasing nodules can be explained as being due to its contained carbohydrate material enabling the bacteria to multiply in the soil. It seems probable that the pentosans and starches in the straw, being readily attacked by bacteria, are the principal sources of energy. This view is consistent with the finding in Experiment 1 that rotted straw did not appreciably increase the nodule numbers. Fresh straw should therefore produce greater effect in stimulating nodule formation than straw that has been mixed in the soil some time and has lost its more easily decomposed constituents before the bacteria are added. The following experiment was made to test this

Table III. *Pot experiment with Vicia faba L.*

Set	Manurial treatment per pot	Dry weight of tops and standard errors (gm.)	Nodule numbers and standard errors
Series A. Straw and phosphate added at time of sowing:			
1	Unmanured	19.6 ± 1.12	234.8 ± 19.6
2	3 gm. CaH <sub>4</sub> (PO <sub>4</sub> ) <sub>2</sub> + 2H <sub>2</sub> O	25.3 ± 1.02	302.0 ± 36.6
3	8 oz. chaff	22.4 ± 2.05	636.5 ± 45.4
4	3 gm. CaH <sub>4</sub> (PO <sub>4</sub> ) <sub>2</sub> + 2H <sub>2</sub> O + 8 oz. chaff	33.7 ± 1.28	866.2 ± 47.1
Series B. Straw and phosphate added one month before sowing:			
5	Unmanured	17.9 ± 0.96	254.5 ± 16.0
6	3 gm. CaH <sub>4</sub> (PO <sub>4</sub> ) <sub>2</sub> + 2H <sub>2</sub> O	18.0 ± 1.62	267.8 ± 20.8
7	8 oz. chaff	17.3 ± 1.97	494.5 ± 54.9
8	3 gm. CaH <sub>4</sub> (PO <sub>4</sub> ) <sub>2</sub> + 2H <sub>2</sub> O + 8 oz. chaff	24.5 ± 2.83	725.0 ± 22.9

point and to confirm the results of Experiment 2 with a different plant. Pots containing 22 lb. of soil-sand mixture were given the manurial treatments shown in Table III. Two series, each having five parallel pots of each treatment, were run; in one series the manurial dressings were

<sup>1</sup> In comparing the mean yields of top in sets 3 and 7,  $t=0.73$ , giving a value of  $P$  lying between 0.5 and 0.4, whereas between sets 2 and 6,  $t=2.9$ ,  $P=0.02$ , and between sets 4 and 8,  $t=2.97$ ,  $P=0.02$ .

added at the time of sowing and inoculation, while, in the other, they were added one month before this, the soil being kept moist. Three broad beans (*Vicia faba* L.) were grown in each pot and inoculated at the time of sowing with a thick suspension of the *Vicia* nodule organism. The results, after three months' growth in a glasshouse, are shown in Table III.

The chaff produced a large increase in nodules, the numbers of which, as in the last experiment, were greater where phosphate is also present. The straw had more influence on nodule numbers when applied fresh at the time of sowing and inoculation than when applied one month before. The freshly applied chaff and phosphate greatly increased the yield when combined, the phosphate alone produced a smaller, but significant increase and the chaff alone an increase whose significance is doubtful. When added one month before sowing and inoculation, the chaff and phosphate significantly increased the yield only where both were present, and then to a smaller extent than when added fresh at the time of sowing<sup>1</sup>. This experiment thus indicates that the more quickly decomposed fractions of the straw are effective in increasing nodule numbers and yield.

Table IV. *Plot experiment on Little Hoos Field, Rothamsted.*

Plot	Treatment in quantities per acre	Yield off 1/100 acre plots in lb.			
		1923. Beans, pods and straw		1924. Wheat, grain and straw	
		Means		Means	
1	No manure	28		21	
6		29.5	28.75	24.5	24.88
11		30		23.5	
16		27.5		30.5	
2	5 tons chaff	33.5		30.5	
7		29	33.33	24	29.17
12		37.5		33	
3	400 lb. superphosphate	27		20	
8		29	27.83	28	24.83
13		27.5		26.5	
4	5 tons chaff + 400 lb. superphosphate	39		33.5	
9		31.5	34.66	23	30.33
14		33.5		34.5	
5	400 lb. superphosphate +	36		23	
10	200 lb. sulphate of	41.5	36.5	36	29.33
15	potash	32		29	

The pot experiments described above indicate that the harmful effects of incorporating fresh straw into the soil do not show on a succeeding

<sup>1</sup> In comparing the means of sets 1 and 2,  $t=3.75$ , giving  $P$  a value of 0.01. In comparing sets 1 and 3,  $t=1.44$ , the value of  $P$  lying between 0.2 and 0.1. For sets 5 and 8,  $t=2.54$ ,  $P$  having a value between 0.05 and 0.02.

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crop of beans or soy beans that is supplied with necessary nodule organisms. It seems possible that this principle might be made use of under field conditions to enable fresh straw to be ploughed in without harmful results. In order to test the action of straw manuring under field conditions, the following experiment was made on Little Hoos Field. The treatments shown in Table IV were tested in 1/100 acre plots. Three plots of each treatment and four unmanured plots were laid down in a randomised arrangement. Beans were sown in April 1923 and reaped in September, and the following season wheat was grown on the same ground. The yield of the two crops is shown in Table IV. The superphosphate alone has not increased the crop, chaff has appreciably increased both the bean crop and the succeeding wheat crop. This effect can also be produced by means of superphosphate + sulphate of potash.

### DISCUSSION.

The ploughing in of unrotted straw produces physical and chemical changes in the soil and also influences the micro-organic population. It has a marked effect in lightening a heavy soil and improving its aeration. There is some evidence that fresh straw contains or produces substances that may be directly toxic to plants but that this factor is of no importance in clay soil(4). The most important chemical changes produced by fresh straw result from the fact that it supplies readily available carbon compounds which are utilised by the soil micro-organisms, so that these multiply rapidly and assimilate the soil nitrates in competition with the crop plants. These effects have been studied by numerous workers (see Murray(5) and Martin(6)). That a similar loss of nitrates takes place under the conditions of pot culture obtaining in the experiments described is shown by the following observations. Two sets of duplicate pots similar to those used in Experiment 1 were filled, the first set with soil-sand mixture only, the second with this mixture plus 8 oz. of chaff. The pots were kept in a greenhouse and maintained at a water content of 14 per cent. and, after one, two and three months, the nitrate, water soluble  $P_2O_5$  and water soluble  $K_2O$  were determined. The results are shown in Fig. 1. The straw caused a considerable loss of nitrates, while the soluble potash was increased, in the first three months after its incorporation with the soil.

Where the succeeding crop is a legume, supplied with its nitrogen-fixing bacteria, it was reasonable to suppose that no loss of yield would result from the depletion of the soil nitrates produced by the straw. The

experiments described above show that in the case of inoculated soy beans and broad beans there was in fact no loss of yield from this cause, and that with the latter, where additional phosphate was also supplied,

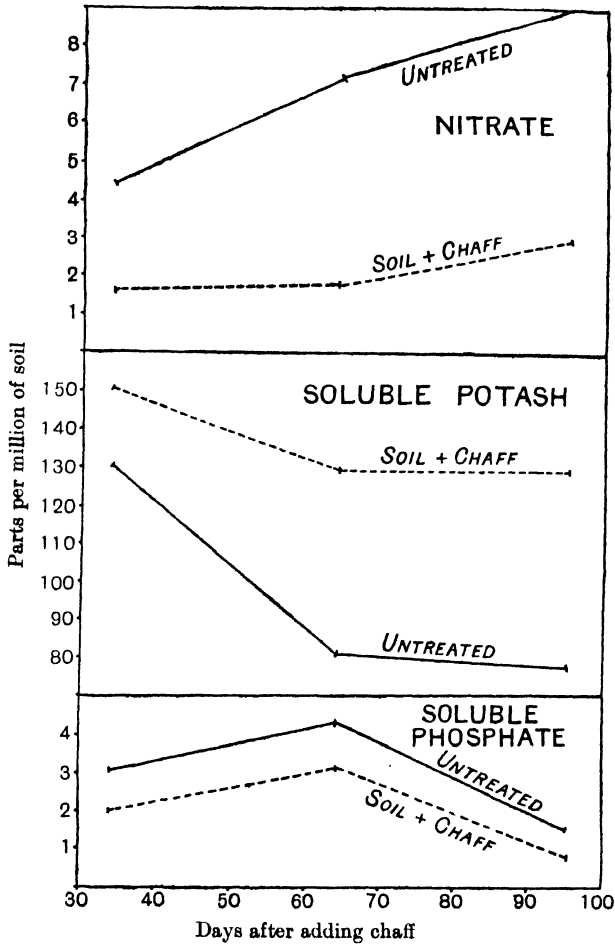


Fig. 1. Effect of chaff on the nitrate and on the water soluble potash and phosphate in pot soil.

there was an actual increase in yield due to the straw. This increase was associated with the development of a considerably greater number of nodules in the presence of the straw.

The increase in nodule numbers by the straw would seem to be explained by the fact that the straw can be utilised as a source of food supply by the nodule organism in the soil. (See Appendix.)

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The initially harmful effect of ploughing in fresh straw has deterred its use, although the benefits eventually resulting from the increase in soil organic matter are recognised. By the use of a bean crop, following its application, this harmful action may perhaps be avoided and the crop actually benefited by the straw. It seems possible that further experiments along this line on different soils and with various legumes may enable the humus content of heavy soils to be raised by straw manuring without temporary loss in crop.

### APPENDIX.

#### THE INFLUENCE OF FRESH CHAFF AND OF CALCIUM PHOSPHATE ON THE MULTIPLICATION OF THE NODULE ORGANISM IN SOIL.

By P. H. H. GRAY.

In order to test the effect of chaff and of phosphate upon the multiplication of the nodule organism in soil, the following experiment was carried out.

One hundred grams of moist sieved soil from the unmanured plot on Hoos Field were placed in each of four Erlenmeyer flasks and the following substances were added.

Flask *A*: 2 gm. of chopped oat straw.

Flask *B*: 0.025 gm. of  $\text{CaH}_4(\text{PO}_4)_2 + 2\text{H}_2\text{O}$ .

Flask *C*: 2 gm. of chopped oat straw + 0.025 gm.  $\text{CaH}_4(\text{PO}_4)_2 + 2\text{H}_2\text{O}$ .

Flask *D*: No addition.

The flasks were plugged with cotton wool, sterilised in the autoclave at 15 lb. pressure for half an hour and inoculated with a pure culture of the lucerne nodule organism, six days old, by adding to each flask four drops from a suspension of the culture in physiological salt solution. Bacterial numbers were estimated at intervals by making plate counts of the soil on an agar medium containing mineral salts, sucrose and extract of lucerne roots. The growth of the organisms is shown in Fig. 2, where each point represents the mean count from four parallel plates.

The chaff has greatly increased the bacterial numbers whether added alone or with phosphate. The phosphate has stimulated multiplication at the commencement, especially where added together with chaff.

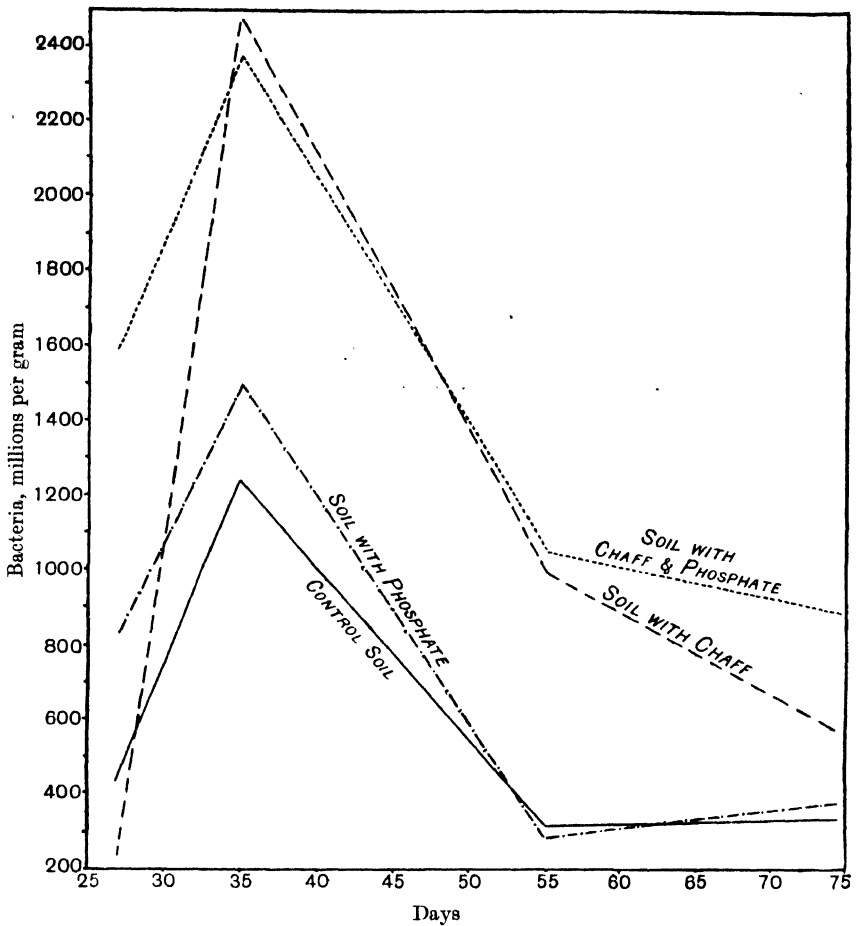


Fig. 2. Growth of the lucerne nodule organism in sterilised soil with and without chaff and  $\text{CaH}_4(\text{PO}_4)_2 + 2\text{H}_2\text{O}$ .

#### SUMMARY AND ABSTRACT.

1. In pot experiments with *Glycine hispida* and *Vicia faba* L., fresh chaff incorporated with the soil caused a significant increase in the number of nodules produced on inoculated plants, this increase being augmented by the further addition of phosphates.

2. Fresh chaff, added at the time of sowing and inoculation, had more effect than chaff which was allowed to decompose in the soil for a month.

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3. Fresh chaff increases the multiplication of the nodule organism in sterilised soil.

4. In soy beans without nodules, the chaff depressed the growth of the tops, but this depression did not occur either with soy or broad beans where nodules were present.

5. In a field experiment made at Rothamsted, chaff, freshly ploughed in, increased the growth of broad beans and also of wheat sown the next season on the same ground.

### REFERENCES.

- (1) RUSSELL, E. J. *J. Board Agric.* (1919), **26**, 124.
- (2) HUTCHINSON, H. B. and MILLER, N. J. H. *Centr. f. Bakt.* (1911), Abt. 2, **30**, 513.
- (3) FISHER, R. A. *Statistical Methods for Research Workers* (1925) (Oliver and Boyd), p. 109.
- (4) COLLINSON, R. C. and CONN, H. J. *New York State Agric. Exp. Sta.* (1925) (Geneva), *Techn. Bull.* 114.
- (5) MURRAY, T. J. *Soil Sci.* (1921), **12**, 233.
- (6) MARTIN, T. L. *Soil Sci.* (1925), **20**, 159.

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# THE "INOCULATION" OF LUCERNE (*MEDICAGO SATIVA*, L.) IN GREAT BRITAIN.

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(With Four Text-figures.)

## 1. INTRODUCTION.

THE discovery in 1884-1886 that the fixation of nitrogen by leguminous crops was due to the infection of their roots by certain bacteria, quickly led to attempts to supply the crop with the necessary organisms. The earlier trials were made at Bremen Experimental Station in 1887 and two years later by Atwater and Woods in Connecticut(1). They consisted in treating the ground sown with a particular legume crop by spreading old soil from a field on which the same crop had been grown. This method has since become a recognised practice in many places with certain legume crops. Considerable quantities of soil, say from 300 to 500 pounds per acre, are needed to ensure successful infection(2), so that the expense of transportation becomes prohibitive where a crop is being introduced into a new district. Moreover there is some danger of introducing plant diseases with the soil. These considerations led to the idea of treating the seed with pure cultures of the nodule bacteria, a process that has come to be known as "inoculation." The earliest experiments with pure cultures were made in Germany by Nobbe in 1896(3) and in Alabama, U.S.A., by Duggar (4) in 1898. Although the experiments were at first hopeful, very variable results were obtained by later workers. An attempt was made to introduce legume "inoculation" into farming practice before the life-history of the organism and its relation to the host plant had been investigated, and while the methods for the preparation and application of pure cultures to the seed were still very inadequate.

It is now known that the nodule bacteria can be divided into a number of physiological groups each of which can normally infect only a small group of legume species(5). The nodule organisms will survive for a great many years in suitable soil(6), and consequently a legume crop that is either indigenous or has been frequently grown in a district will usually find the soil already infected with its own variety of the organism. On the other hand a recently introduced legume crop will

probably find its own specific variety of the bacteria absent from the soil, whilst other varieties cannot normally produce nodules upon it. It is therefore in the case of legume crops introduced into new districts that inoculation is mainly required, and it is with such crops that most successful results have been obtained.

By far the most important of such crops is Lucerne (*Medicago sativa*), the cultivation of which has spread over large areas in all parts of the world during the last hundred years. Indigenous to Western Asia, lucerne was introduced into Southern Europe in classical times and its cultivation gradually spread northwards, reaching England in the middle of the seventeenth century. The seed was on sale in London in 1651 (7). Since then the cultivation of the crop has been mainly confined to the south-eastern quarter of England. The soil in this region has by this time become infected with the lucerne variety of the nodule organism. Commercial samples of lucerne seed are frequently contaminated with the organism. Thus it has been found at Rothamsted that when a sample of lucerne seed is sown in sterilised sand, the resulting seedlings develop a small number of nodules although under similar circumstances no nodules appear if the outer surface of the seeds is sterilised with mercury chloride. It is thus probable that the establishment of the lucerne organism in the soil of the south-eastern counties has been gradually brought about by its chance introduction in samples of the seed.

In the remaining districts of Great Britain, where lucerne is only very occasionally grown, it frequently fails and in many such cases no nodules are developed. There seemed a possibility, therefore, that a dominant cause of failure in these districts was the absence of the lucerne nodule organism from the soil, the number of bacteria introduced by chance with the seed being insufficient to produce any result in a single sowing. If this were so it seemed that the area of successful lucerne cultivation might be extended by introducing the organisms with the seed.

Various attempts to improve lucerne growth by inoculation had been made in past years. Those at Rothamsted, where the soil already contains the lucerne organism, did not give beneficial results. A success, however, was obtained by Wright (8) at the West of Scotland Agricultural College in an experiment commenced in 1905 and carried on for five years.

Of recent years our increasing knowledge of the nodule organism has led to considerable improvements in the technique of inoculation. It is now known (9) that the nodule bacteria infecting lucerne are divisible into distinct groups which differ in their nitrogen fixing efficiency and can also be distinguished by serological tests and by their growth on

media. It is therefore important that an efficient variety be used. The strain employed in the field trials described below belongs to the more efficient of these groups and was obtained from the Danish State Laboratory, Lyngby, Copenhagen. The strain was compared by Cunningham<sup>(10)</sup> with one obtained from the United States Department of Agriculture. In a pot experiment, lucerne inoculated with it showed an increase in air dried crop of 81.5 per cent. over the uninoculated, while the American strain produced an increase of 16.3 per cent., both after 3 months' growth. In the earlier work on inoculation a frequent cause of failure was the death or loss of virulence of bacteria in the laboratory. This can now be overcome by storing cultures of the organisms in sterilised soil, a method suggested by Simon<sup>(11)</sup>. For the preparation of the cultures for issue to the farmers, on the other hand, a medium is needed upon which rapid, vigorous growth will be induced so that fresh cultures can be produced at as short notice as possible. P. H. H. Gray at Rothamsted has produced an agar medium containing an extract of lucerne roots upon which abundant growth can be obtained after four days' incubation. Finally, recent knowledge of the life-cycle of the nodule organisms in the soil has resulted in the development of a method for applying them to the seed, that results in the production of motile forms in the soil, thus increasing the chances of root infection. This method, developed by Thornton and Gangulee<sup>(12)</sup>, has been used throughout the field trials described below.

The better chances of success with inoculation due to increased knowledge and to improvements in technique, therefore, made it worth while to give the process a critical test in this country. For this purpose the lucerne crop was particularly suitable, since it was possible to test inoculation in the soils of the south-eastern counties where the lucerne organism was already established, and in other districts where the organism was thought to be generally absent from the soil. Since it was in the latter districts that beneficial results from inoculation were to be expected, the problem was essentially bound up with the geographical distribution of the lucerne organism in British soils. The information obtainable from isolated experiments could not therefore be applied to other areas, since it was probable that inoculation would be beneficial in some districts and not in others. It was necessary therefore to make trials in a large number of localities in order to see in what districts inoculation would be effective. There was also the hope that new areas might be found where, by means of inoculation, the successful growth of this valuable forage crop might be possible. Through the generosity

of the Royal Agricultural Society in providing grants of money an extensive series of experiments in localities covering the greater part of Great Britain have been organised during the past five years. These experiments have been carried out at Agricultural Colleges, Farm Institutes and especially by private landowners, often under the supervision of the local agricultural officer. Careful experiments from so many widely scattered localities were rendered possible only by the keen and generous cooperation of these many experimenters, who have spared neither the trouble nor the expense involved and to whom the successful results are largely due.

## 2. PLAN OF THE EXPERIMENTS.

In order to make the results from different localities comparable the experiments were carried out as far as possible on the following general plan. Eleven plots usually of  $1/5$ th acre were laid down, six being sown with untreated and five, arranged alternatively with them, with inoculated seed. The plots in the earlier trials were separated by 2-3 foot paths, but these were later omitted on account of the difficulty in keeping them weeded. The sowing and subsequent operations on the plots were performed on the untreated plots first, in order to lessen their accidental infection with inoculated soil. The time of sowing was necessarily determined by local circumstances. In 1926 a number of experiments were laid down with the object of comparing the spring and summer sown lucerne, as well as the effect of inoculation. These trials which are described below consist of 12 plots, half of which were sown with inoculated and half with untreated seed. In addition to the above experiments, several smaller trials have been made in various places.

The cultures used for inoculation were grown in test tubes on slopes of the lucerne root-extract agar medium previously mentioned. The cultures were incubated at  $25^{\circ}$  C. for 4 to 7 days before issue to the experimenter and were used within 6 weeks of their preparation. In treating the seed the method of Thornton and Gangulee<sup>(12)</sup> was employed. The bacteria were applied at the rate of 1 culture to 7 lb. of seed. Experiments now in progress suggest that this quantity of seed can be greatly increased with little loss of effect. The cultures were suspended in a quantity of skim milk that would wet the whole mass of seed. It was found that  $\frac{1}{2}$  pint to 7 lb. of seed would just do this. Before adding the culture, calcium di-acid phosphate,  $\text{CaH}_4(\text{PO}_4)_2 + 2\text{H}_2\text{O}$ , was dissolved in the milk to make a 0.1 per cent. solution, the correct amount of this salt being sent out with the cultures. The milk containing the

Table I. *List of experiments to test lucerne inoculation.*

No. on map	Experimenter	Trial under the supervision of	Soil type	Date of sowing	Cover crop	No. of plots	Size of plots (acre)
<b>A. Western area:</b>							
1	F. Ballard, Maybole, Colwall, Hereford	J. L. Evans	Light limestone	May 1926	No	4	$\frac{1}{4}$ to 1
2	R. T. Board, Merthyr Mawr, Bridgend, Glamorgan	J. D. Davidson and H. Rhys Williams	Light gravel	May 1924	No	11	1/5th
3	R. V. Bradburn, Stanmore Farm, Bridg-north	—	Light	May 1924	No	11	1/5th
4	Col. E. P. Brassey, Upper Slaughter, Gloucestershire	G. H. Hollingworth and C. Comely	Shallow limestone	May 1925	Yes	11	1/5th
5	A. T. Cake, Higher Came, Dorset	T. R. Ferris and J. A. Robotham	Light chalk loam	May 1926	No	11	1/5th
6	Rt. Hon. Lord Clinton, Rolle Estate, Exmouth	—	Red sandstone	June 1925	No	4	$\frac{1}{2}$
7	G. H. Johnstone, Tregoose, Gramppound Road, Cornwall	A. Gregg	Clay shale	May 1925	No	11	$\frac{1}{4}$
8	J. H. Malcom, Ty Gwyn, Clydach, Swansea	J. D. Davidson and H. Rhys Williams	Poor grit soil	May 1924	No	11	1/5th
9	A. S. Matthias, Llangwarren, Pembrokeshire	—	Shale	April 1925	Yes	4	$\frac{1}{4}$
10	Seale Hayne Agric. College, Newton Abbot	T. J. Shaw	Shallow shale	April 1924	Yes	11	1/5th
11	G. Sheaf, Honeybourne, Gloucestershire	G. H. Hollingworth and C. Comely	Heavy clay	May 1925	No	11	1/5th
12	W. Smith, Cogan Hall, Penarth	J. D. Davidson and H. Rhys Williams	Clay loam	June 1924	No	11	1/5th
13	Welsh Plant Breeding Station, Aberystwyth	Prof. R. G. Stapledon	Poor shallow shale	May 1925	No	21	1/24th
<b>B. Northern area:</b>							
14	J. D. Johnstone, Eden Lacey, Lazonby	Principal R. Lindsey Robb	Sandy loam	June 1926	No	11	1/5th
15	W. Low, Balmakewan, Marykirk	Prof. J. Hendrick	Red sandstone	May 1924	No	11	1/5th
16	J. W. McGilivray, Aberdeen	—	Loam over clay	May 1927	No	4	1/16th
17	Pennel and Sons, Welton, Lincoln	—	Clay	June 1926	No	8	1/8th
18	E. Abel Smith, Longhills, Lincoln	—	Gravel	April 1924	No	11	1/5th
19	W. R. Strickland, Baness, Catterick	—	Clay	June 1925	No	10	1/10th
20	J. Walker, Houghall, Durham	—	Gravel	June 1925	No	8	1/8th

C. *Central area:*

21	A. T. Carr, Turner's Court, Wallingford	—	Chalk	Aug. 1925	No	11	1/5th
22	C. C. Edmunds, Mentmore Estate, Leighton Buzzard	—	Heavy clay	April 1924	No	11	1/5th
23	C. Barwell Field, Bowmans, Colney, Herts.	—	Gravel loam	Aug. 1924	No	11	1/5th
24	Herts. Institute of Agriculture, Oaklands, St Albans	J. Hunter Smith and H. Rhys Williams	Clay with flints	April 1925	(Yes {No	10 11	1/5th 1/5th
25	W. Keevil, Berhill, Calne, Wiltshire	—	Oolite limestone	May 1924	No	11	1/5th
26	W. Lawson, West Sussex County Council Farm, Chichester	—	Clay	May 1926	No	11	1/10th
27	Col. C. Lyon, Appleton Hall, Cheshire	W. B. Mercer	Clay loam	July 1925	No	6	1/10th
28	Col. F. H. N. Meynell, Hoar Cross, Burton-on-Trent	—	Gravel	June 1925	No	6	1/6th
29	G. W. Olive, Dauntsey School, Wiltshire	—	Sandy loam	April 1924	No	8	1/12th
30	Studley College, Warwick	Miss Melville Jackson	Loam	July 1925	No	11	1/10th
31	Woburn Experimental Station	Dr J. A. Voelcker	Sandy	July 1927	No	11	1/5th
32	A. A. White, Ardley Fields, Bicester	G. R. Bland	Limestone	July 1926	No	10	1/5th
D. <i>South-eastern area:</i>							
33	Australian Farms Training College, Lynford Hall, Norfolk	Principal H. H. Potts	Sandy loam	Sept. 1926	No	11	1/5th
34	A. E. Meeson, Eastling, Faversham	—	Clay with flints	Aug. 1924	No	11	1/5th
35	A. W. Oldershaw, Tunstall Heath, Suffolk	—	Light sand	July 1926	No	6	1
<i>Spring and summer sown trials:</i>							
36	A. Clarke and Sons, Chiselborough, Somerset	W. D. Hay and J. D. Dallas	Sandy	May and July 1926	Spring sowing with cover crop	12	1/10th
37	County School, Welshpool, Montgomery	C. Harrison	Shale	—	"	12	1/40th
38	C. H. Roberts, Boothby, Brampton	H. W. Cousins	Sandy	April and July 1926	"	12	1/8th
39	Rothamsted Experimental Station	—	Clay with flints	April and July 1926	"	12	1/10th

cultures in suspension was poured on to the seed and thoroughly mixed with it, until every seed was wetted. The seed was then spread out in the shade and allowed to dry. It was found to be very important that the seed should be completely dried, otherwise difficulty was experienced owing to the seed clogging the drill. The use of skim instead of whole milk greatly shortens the time of drying.

Since little was known as to the suitability of many districts for growing inoculated lucerne it was inevitable that at a number of centres the crop should fail. The causes of crop failure are discussed below, but in discussing the effects of inoculation only those trials are considered which are still running or which were continued for long enough for the effect of the inoculation to be determined. A list of these centres is given in Table I.

### 3. EFFECT OF TREATMENT IN DIFFERENT PARTS OF GREAT BRITAIN.

The map, Fig. 1, shows the distribution of the trials that are here discussed, indicating in which of them an improved growth as a result of inoculation has been observed.

A summary of the yield and analysis results so far obtained from the trials is shown in Tables II, III and IV. Wherever possible the whole crop from each plot was separately weighed, but in some cases, as shown in the table, a cutting from an equal area on each plot was taken. The figures give mean weights and analyses from the parallel plots.

There is no evidence that the response to inoculation is related in any way to soil or to climatic conditions. As was expected, however, the effects produced by inoculation do vary in different districts. It is thus convenient to divide the country into areas and to consider the experiments in each area separately.

A. *The south-western area.* This includes Wales and the counties of Shropshire, Hereford, Gloucester, Somerset, Dorset, Devon, and Cornwall. A marked benefit from inoculation has been observed in almost all experiments in this area (Table II). The effect has usually shown itself quite early in the life of the seedling plant and has persisted throughout the trial save where there has been evidence that the untreated plots have become infected. In Wales, Mr Harrison obtained a 58.4 per cent. increase in crop from inoculation, while, in a trial made by Prof. Stapledon at the Welsh Plant Breeding Station, Aberystwyth, the inoculated plots bore a stronger and deeper coloured growth during the first year. In this latter trial, however, the untreated plots became infected before weighings were taken. The experiments in Glamorgan and the small

Table II. *Yield and nitrogen contents of the crops. South-western area.*

Year of sowing	Month and year of cutting	Weights obtained from			Whether weighed green or as hay	Yield in cwt. per acre		Per-centage increase over un-treated	Percentage nitrogen content		Cwts. of nitrogen per acre in crop	
		No. of plots	Area per plot in sq. yds.			Inocu-lated	Un-treated		Inocu-lated	Un-treated	Inocu-lated	Un-treated
1925	June 1926	11	968		Hay	23.7	14.5	63.4	2.77	2.59	0.66	0.38
	Sept. 1926	11	968		Green	40.2	14.2	183.1	—	—	—	—
	June 1927	11	82		Hay	109.3	32.5	236.1	2.53	2.1	2.77	0.68
	Sept. 1927	11	67.5		Green	71.7	38.4	86.7	—	—	—	—
	June 1928	11	73		Green	91.7	62.0	47.9	2.97	2.02	2.72	1.75
	Aug. 1928	11	41		Green	49.5	33.0	52.7	—	—	—	—
1926	June 1927	11	66		Hay	20.9	17.0	23.0	—	—	—	—
1925	July 1926	11	605		Semi-dry	53.0	34.0	55.9	3.91	3.13	2.07	1.06
	July 1927	11	605		Semi-dry	52.0	50.0	4.0	3.38	3.24	1.76	1.62
1925	Sept. 1925	8	80		Green	44.25	35.25	25.5	—	—	—	—
	July 1926	4	40		Semi-dry	61.5	41.9	46.8	3.4	3.3	2.09	1.38
	Sept. 1927	9	48		Semi-dry	38.2	39.3	-2.8	4.1	3.6	1.56	1.41
	Sept. 1925	11	145		Green	21.8	7.8	179.4	3.94	2.84	0.86	0.22
1925	Aug. 1926	11	108		Green	44.7	32.5	37.5	3.34	3.3	1.49	1.07
	June 1927	11	36		Green	61.2	51.6	12.7	2.54	2.36	1.55	1.22
	July 1926	12	20		Green	192.7	174.2	10.6	—	—	—	—
	July 1927	12	20		Green	118.4	112.4	5.3	—	—	—	—
1926	Oct. 1926	6	30		Green	37.5	33.8	10.9	2.57	2.62	0.96	0.88
	July 1927	12	484		Green	81.75	66.8	22.4	4.13	4.12	3.38	2.75
1926	July 1927	12	121		Green	87.1	55.0	58.4	2.55	2.66	2.22	1.46
	Oct. 1927	12	121		Green	43.9	33.3	31.8	2.85	2.9	1.25	0.97

trial made by Mr Matthias in Pembrokeshire all showed a visible difference from treatment. A very marked effect was also observed in Mr Braburn's trial in Shropshire and in Mr Ballard's in Herefordshire.

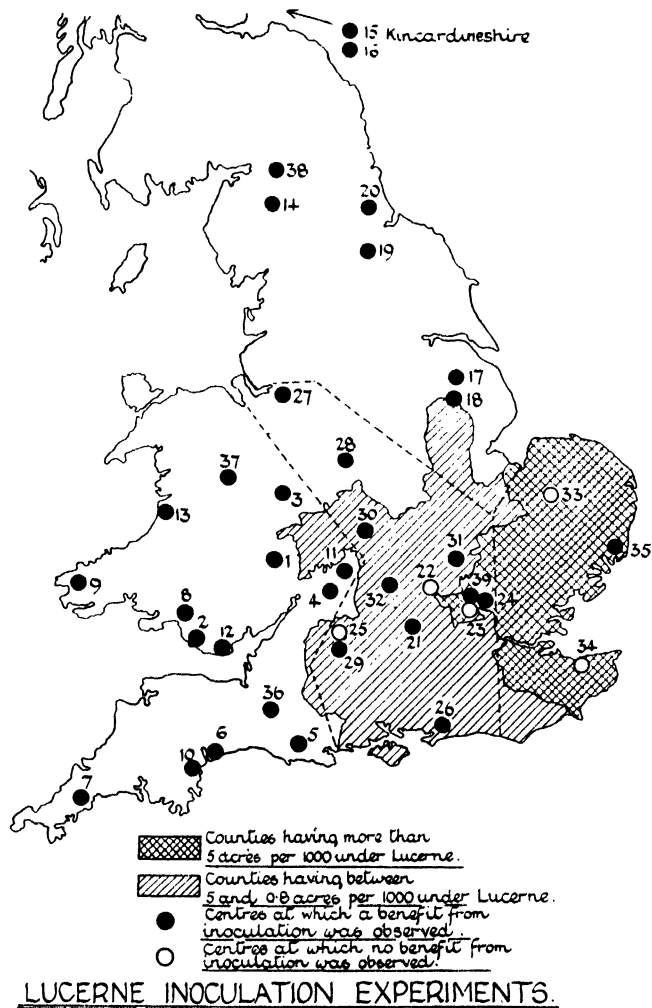


Fig. 1.

The most striking results, however, have been obtained from Gloucester, increases of 236.1 per cent. and 179.4 per cent. in the yield having been recorded from Colonel Brassey's and Mr Sheaf's experiments. Examination of the roots of the untreated plants on Colonel Brassey's land showed an almost complete absence of nodules. Thus 150 untreated

Table III. *Yields and nitrogen contents of the crops. Northern area.*

Experimenter	Year of sowing	Month and year of cutting	Weights obtained from			Whether green or as hay	Yield in cwts. per acre		Percentage increase over untreated	Percentage nitrogen content		Cwts. of nitrogen per acre in crop	
			No. of plots	Area per plot in sq. yds.	Area per weighed from		Inoculated	Untreated		Inoculated	Untreated	Inoculated	Untreated
W. Low, Kincardineshire	1924	Oct. 1924	22*	484	Green	Green	83.5	75.8	10.2	—	—	—	—
		July 1925	22	484	Hay	Hay	48.3	34.3	40.9	—	—	—	—
		Sept. 1925	22	484	Green	Green	60.3	51.3	17.5	—	—	—	—
		July 1926	22	484	Hay	Hay	41.5	37.5	10.7	—	—	—	—
		Sept. 1926	22	484	Green	Green	63.75	61.25	4.1	—	—	—	—
		Aug. 1927	22	484	Hay	Hay	58.5	55.5	5.4	—	—	—	—
		Sept. 1927	22	484	Green	Green	33.5	32.0	4.7	—	—	—	—
		July 1927	8	605	Green	Green	151.5	125.5	20.7	2.64	2.56	3.99	3.2
Pennell and Sons, Lincoln	1925	July 1926	11	40	Green	Green	100.0	62.1	61.0	3.4	2.2	3.4	1.37
		Sept. 1925	8	302	Green	Green	62.5	53.5	16.8	—	—	—	—
		Sept. 1926	6	605	—	—	173.0	87.4	98.0	—	—	—	—

\* In Mr Low's experiment each plot was divided longitudinally and the crop from each half separately weighed.

lucerne plants examined bore only 22 nodules, whereas 100 inoculated plants from adjacent plots bore 341 nodules. It is thus probable that the lucerne organism is absent from this soil, accidental transfer of bacteria in the seed sample being sufficient to account for the small number of nodules on the control plants.

In Somerset, Messrs Clarke and Sons' experiment showed an increase of 22.4 per cent. in 1927. In Devon, Lord Clinton's trial showed an increase of 55.9 per cent. in 1926, and in Cornwall, Mr Johnstone's trial showed an increase of 46.8 per cent. in the same year. At Seale Hayne Agricultural College, the inoculation produced a visible improvement, but the crop failed owing to the shallowness of the soil. In Dorset, Mr A. T. Cake obtained an increase of 23.0 per cent. from inoculation.

B. *Northern area.* In the northern half of England and in Scotland, the experiments have invariably shown a benefit from inoculation as in the south-western area (Table III). In Lincolnshire, Messrs Pennell and Sons obtained an increase of 20.7 per cent. in yield from the treatment, and much of the untreated lucerne died and was replaced by rye grass, while Mr Abel Smith's plots showed a visible effect. In Mr Strickland's experiment at Catterick the treatment increased the yield by 61 per cent., and in the experiment at Houghall Farm, Durham, by 98 per cent. At the two centres in Cumberland the inoculated plots were visibly stronger from the first, indeed, in Mr Roberts' experiment, only the inoculated lucerne grew. In Scotland, Mr Low's trial near Montrose showed an increase of 40.9 per cent. in 1925. A number of trials organised by Cunningham<sup>(10)</sup> near Edinburgh and Wright's trial<sup>(8)</sup> at the West of Scotland Agricultural College in 1908, showed that in other parts of Scotland, also, inoculation produces a striking effect.

C. *The central area.* This forms a broad zone separating the western area discussed from eastern lucerne growing area. Experiments in the counties of Cheshire, Staffordshire, Warwick, Berkshire, Hertford, Wiltshire, Hampshire, and West Sussex mostly show an increased growth from inoculation which, though sometimes considerable at a certain stage in the trial, is transient (Table IV). Table V shows in several trials how soon after sowing the effect from inoculation became visible and for how long this effect remained.

In plots sown without a cover crop, after periods varying from 6 to 14 months the growth of the untreated lucerne has caught up that of the inoculated plants. The trial at Rothamsted is typical. It is known as a result of numerous experiments that the soil at Rothamsted contains the lucerne nodule organism, but in such small numbers that

Table IV. *Yields and nitrogen contents of the crops.*  
*Central and south-eastern areas.*

Year of sowing	Month and year of cutting	Experimenters	Weights obtained from		Whether green or as hay	Yield in cwt., per acre		Percentage increase over untreated	Percentage nitrogen content		Cwts. of nitrogen per acre in crop	
			No. of plots	Area per plot in sq. yds.		Inoculated	Untreated		Inoculated	Untreated	Inoculated	Untreated
1925	July 1926	Central area: A. T. Carr, Berks. Hertfordshire Institute of Agriculture	11	30	Green	7.8	6.1	27.9	—	—	—	—
	May 1926		5	968	Hay	219	167.6	30.0	—	—	—	—
	July 1926		5	968	Hay	178.5	147.8	20.8	—	—	—	—
	Sept. 1926		5	968	Hay	44.6	37.6	18.6	—	—	—	—
	May 1927		5	968	Green	37.4	34.1	9.5	—	—	—	—
	July 1927		5	968	Green	28.1	27.2	3.3	—	—	—	—
	Sept. 1927		5	968	Green	12.05	12.2	-0.8	—	—	—	—
1926	May 1927	W. Lawson, Sussex	11	968	Green	211.6	187.2	13.4	3.56	3.36	7.5	6.3
	July 1927		11	968	Green	177.5	166.5	6.6	—	—	—	—
	June 1926		6	242	Green	118.3	110.7	6.9	4.61	3.99	5.45	4.41
1925	Oct. 1925	Col. C. Lyon, Cheshire	6	807	Hay	14.54	14.28	1.8	3.21	2.19	0.47	0.31
1925	Sept. 1925	Col. F. H. N. Meynell, Staffordshire	11	28	—	162.8	120.4	35.2	—	—	—	—
	June 1926		11	3	—	216.9	210.4	3.1	2.93	2.88	6.36	6.06
	June 1927		11	11	—	191.3	180.4	6.0	2.65	2.64	5.07	4.76
	July 1928		11	726	Hay	38.0	30.8	23.4	3.18	1.65	1.21	0.51
1927	June 1927	Woburn Experimental Station, Bedfordshire	11	968	Green	22.8	21.95	3.6	2.99	3.04	0.68	0.67
1926	Aug. 1928	South-eastern area: Australian Farms Training College, Norfolk	11	968	Hay	4.97	5.32	6.6	—	—	—	—
	June 1928		18	30	Green	21.6	12.2	77.5	—	—	—	—

\* In Mr A. W. Oldershaw's trial the weights from the chalked plots alone are included.

the nodules on lucerne plants are greatly increased by inoculation. Thus in a pot experiment with Rothamsted soil the nodules per plant (means of 50 plants) were: inoculated, 118.3; untreated, 46.7; and in a second similar experiment (means of 10 plants): inoculated, 113.1; untreated, 43.8. This increase in nodule numbers appreciably affects the growth.

Relation between number of Nodules and  
Top weights in Lucerne, 10 weeks old.

Each point represents the mean of from  
60 to 90 plants, from a pot culture experiment.

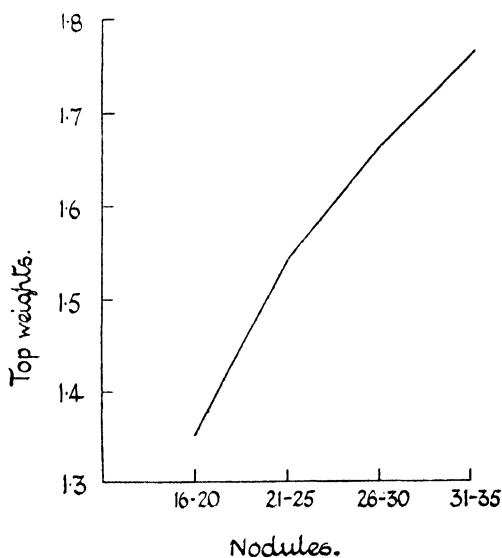


Fig. 2.

In the above pot experiments the increases in top weight of inoculated over control plants were 40.5 per cent. and 16.1 per cent. respectively. Indeed in the early period of growth the top weights and nodule numbers are correlated (Fig. 2). The heavier nodule formation produced by inoculation is thus sufficient to increase the growth of the lucerne in the first few months even in soils that contain a certain number of the lucerne nodule organisms. On clean land and under good conditions the untreated lucerne may recover from its weak start. Since the experiments mentioned in Table V show no evidence of a spread of organisms from the edges of the inoculated plots, this recovery is probably due to the multiplication of the bacteria already present in the soil. Where, however,

Table V.

Experiment	Time before the effect appeared (months)	Cover crop	How long the effect lasted (months)
Rothamsted Experimental Station ... ..	2	No	10
Studley College, Warwick ... ..	2	No	10
Oaklands Farm Institute, St Albans ... ..	2	No	6
		Yes	30
A. A. White, Bicester ... ..	9	No	10
Farm Training Colony, Turners Court, Wallingford	10	No	14
W. Lawson, Sussex ... ..	3	No	12

the young lucerne has to compete with weed infestation or with a cover crop, the inoculation may produce a much greater benefit, since, in this case, a weak young plant will tend to be smothered and, moreover, the weeds or cover crop, by competing for combined nitrogen, force the lucerne to depend upon the nitrogen from its nodules at an earlier stage of growth. The experiment at the Hertfordshire Agricultural Institute, St Albans, affords an instructive example of this. Here, the usual trial consisting of eleven plots of 1/5th acre was laid down, the lucerne being sown on bare ground in April 1925. Adjoining this were a series of plots each of approximately half an acre, in which lucerne alone and in various seeds mixtures was sown in a cover crop of barley. The arrangement of these plots was as shown in Table VI. The lucerne sown without a cover

Table VI. *Lucerne sown under a cover crop of barley at the Hertfordshire Institute of Agriculture.*

*(Trial carried out by Mr J. Hunter Smith.)*

*Cutting made May 1926, 14 months after sowing.*

Plot	Size of plots 0.75 to 1 acre	Yield in cwt. per acre		
		Inoculated	Untreated	Increase
A Lucerne drilled ... ..		232.0	180.0	52.0
B Lucerne drilled—1 lb. per acre wild white clover		233.0	217.0	16.0
C Lucerne drilled—2 lb. per acre cocks foot ...		208.0	133.0	75.0
D Lucerne drilled—2 lb. per acre Italian rye grass		202.0	139.0	63.0
E Lucerne, broadcast ... ..		220.0	169.0	51.0
Mean ... ..		219.0	167.6	51.4

crop showed a visible effect from inoculation which influenced both growth and especially the colour of the plant. This effect became noticeable in June and was very marked in August 1925. During September the untreated plots began to darken in colour uniformly, and by October it was no longer possible to distinguish between the

plots. On the plots sown in a cover crop, on the other hand, the early start due to inoculation produced a more lasting effect. In October the inoculated lucerne was about 6 inches high while scarcely any was visible on the untreated plots. In June 1926 the cover crop plots gave the yields shown in Table VI. Even on these plots, however, the untreated lucerne recovered during 1927 (Table IV). Trials made by Mr Edmunds on the Mentmore Estate, Leighton Buzzard, Mr Barwell Field near London Colney, and by Mr W. Keevil near Calne, showed no effect from inoculation. It seems probable that at these centres the soil contained a population of lucerne bacteria sufficient for the plant's needs. At Woburn Experimental Farm a marked effect from inoculation still persists. The previous acidity of the soil here makes this experiment more comparable with that at Tunstall, Suffolk, which is discussed below.

The experiments in the midlands and south central counties, taken as a whole, indicate that the soils usually contain a sparse population of lucerne bacteria which, if the crop survives long enough, will eventually infect it, but that, in these soils, inoculated lucerne makes a stronger growth in the young stages, an advantage which may produce a lasting effect on the crop where it has to compete with other plants. In this district, therefore, inoculation is advisable as a precaution against unfavourable conditions in the first year and should always be adopted when the lucerne is sown in a cover crop.

*In the south-eastern counties* about a dozen trials were started, the majority of which unfortunately failed owing to the wet summer of 1924, but those that survived during the seedling year did not in this time show any visible improvement from inoculation. In an experiment carried through at the Australian Farms Training College at Lynford Hall, Norfolk, the yield results showed no benefit from the treatment (see Table IV). Over most of the south-eastern area the lucerne organism is apparently present in the soil as is indicated by the success with which uninoculated seed can be grown. An interesting and important exception is afforded by the trial carried out by Mr A. W. Oldershaw, on the County Council Experimental Farm at Tunstall, near Ipswich. This farm is on an area of very light, sour land, having a reaction of about pH 5.4. The trial was made both with the object of testing the value of inoculation and to see whether lucerne could be grown on this land after liming. The experimental plots of inoculated and untreated lucerne were therefore laid across strips left unlimed and limed in various ways. Table VII shows the plan of the trial. The lucerne was sown in July 1926. By the following March no effect from inoculation was visible,

Table VII. *Plan of the experiment at the East Suffolk County Council Farm, Tunstall, showing yields in 1928, as cwt. per acre.*

	Control	Inocu- lated	Control	Inocu- lated	Control	Inocu- lated	Mean yield inocu- lated	Mean yield control
No lime	4.5	5	4.5	5.5	4.5	13	7.5	4.5
Crag	No weights taken							
5 tons per acre								
Chalk	19	26.5	12.5	16	11.5	18.5	20.3	14.3
5 tons per acre								
Chalk	11.5	23	7	18.5	10	18	19.8	9.5
10 tons per acre								
Chalk	12.5	37.5	14	22.5	12.5	24	24.7	13
20 tons per acre								

but only the inoculated plants bore nodules. By the middle of June a very marked effect from inoculation was visible on the limed plots and analysis showed the following nitrogen percentages in tops and roots:

	Inoculated			Control
Tops ...	...	...	2.55	1.31
Roots	...	...	1.63	0.88

In 1928 the first cutting gave the yields shown in Table VII. The experiment shows that even in a region such as East Anglia whose soils generally contain the lucerne organisms, they may be absent where the soil is acid. This conclusion is supported by other workers. Fred and Davenport<sup>(13)</sup> testing seven strains of the lucerne organism found that in laboratory cultures in a liquid medium an acidity greater than  $pH$  5 prevented growth, while Bryan<sup>(14)</sup> found that they were killed by storage for 75 days in soil more acid than  $pH$  5. Joffe<sup>(15)</sup> and Bryan<sup>(16)</sup> found that nodule development on lucerne fell off rapidly as the acidity increased from  $pH$  7 to  $pH$  4 although a few nodules developed at the latter reaction. The dying out of lucerne bacteria in acid soils thus makes inoculation important in these soils after they have been limed. Thus Fred and Gaul<sup>(17)</sup> found that in the acid soils of Wisconsin inoculation was very beneficial especially when combined with liming. This is also the common experience in Denmark.

#### 4. SPREADING OF THE BACTERIA.

In many cases the weight results underestimate effects of "inoculation." This is due to two causes. In the first place the untreated plots tend to become very weedy and the crop weighed often consists largely of weeds. This is for example the case on Col. Brassey's plots where, in 1927, the untreated plots bore only an occasional small plant of lucerne,

the weight of crop being made up entirely of other plants, mostly red clover (see Table II).

In the second place, there have been certain experiments where the control plots have become infected by the migration of the nodule bacteria into them from the inoculated plots. This usually shows itself above ground by the gradual spreading of the deeper green colour from the edges of the inoculated plots. In the case of Mr W. Low's and

MR. W. LOW'S EXPERIMENT

Lessening difference caused by  
infection of the untreated plots

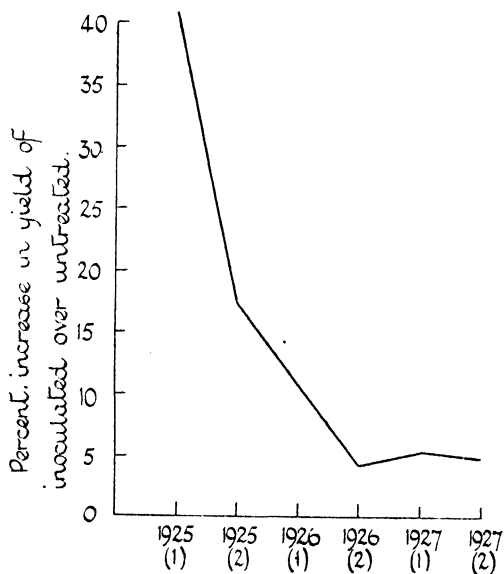


Fig. 3.

Mr G. Sheaf's experiments the whole of the control plots have now become infected. The yields from Mr Low's plots illustrate very clearly the apparent loss of effect from inoculation which is in reality due to the gradual infection of the control plots (Fig. 3).

Observations of the spreading on Mr Sheaf's plots showed that it commenced in the middle of August 1925 and continued until winter set in, the plants in the newly infected areas bearing numerous nodules. No further spreading was noticed until the following June when the spread recommenced and continued during the summer, by the end of which the control plots were almost completely inoculated (Table II).

It is thus probable that the spreading is affected by season, and does not occur except under special conditions of soil moisture and temperature. It is at any rate an exceptional phenomenon, marked spreading having been observed in only six of the experiments. It is not apparently related to soil type, though in two cases it was clearly connected with the wetness of the soil. The migration of the bacteria must have a considerable bearing upon the slow infection of a soil by the chance introduction of small numbers of nodule bacteria in samples of uninoculated seed. The sporadic occurrence of spreading indicates that only under exceptional conditions will a soil become rapidly populated with the organism by such accidental means.

#### 5. NITROGEN CONTENT OF THE CROP.

The inoculation sometimes causes an increase in the nitrogen content of the crop without raising the yield. This has occurred, for example, in Colonel Meynell's experiment (Table IV). More often both the yield and the percentage of nitrogen are increased. In both these cases it would seem that some other limiting factor has prevented the crop from taking full advantage of the increased nitrogen supply. In other cases, as in Mr Lawson's and Messrs Pennell and Sons' trials, there is an increase in the crop but no significant increase in the percentage of nitrogen in it. In such cases the plant has been able to make full use of the nitrogen to increase its bulk.

#### 6. PROSPECTS OF SUCCESS WITH LUCERNE IN THE VARIOUS DISTRICTS.

The better crop obtained by inoculating lucerne in the south-west, west and north of Great Britain raises the question: Can the successful growth of lucerne be extended westwards and northwards by the use of the present improved methods of seed inoculation? During the last two years cultures for inoculation have been sold to about 200 farmers distributed over nearly all parts of England. The success or failure of the crop from inoculated seed has been reported in a considerable number of cases so that we now have a good deal of evidence, apart from that obtained from actual experiments as to chances of getting a crop from inoculated lucerne in various districts. The map, Fig. 4, shows the distribution of successes and failures with inoculated seed sown in 1925, 1926 and 1927. The figure only shows whether a successful crop was obtained from the inoculated seed and does not take into account the growth of untreated lucerne in places where this was sown. The area east of Longitude  $1.5^{\circ}$  and south of Latitude  $53^{\circ}$  may be taken as being

that over which the crop is at present cultivated to an appreciable extent. Within this area inoculated seed gave a successful crop at 35 out of a total of 49 centres (71.4 per cent.). In the remainder of Great Britain where the crop is at present very seldom grown, inoculated seed gave successful crops at 53 out of 78 centres (67.9 per cent.). Thus in

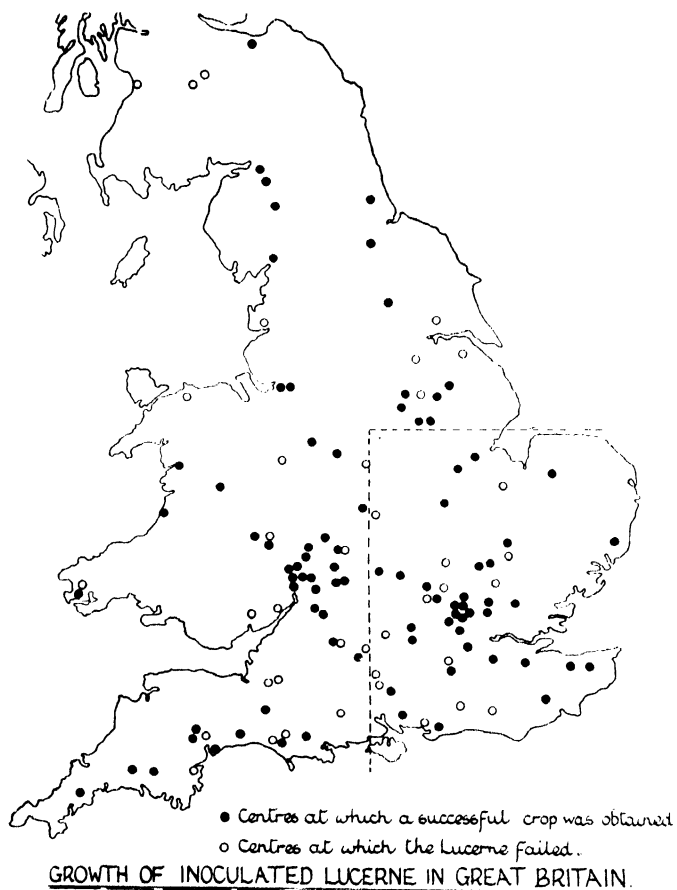


Fig. 4.

spite of the fact that this latter area includes centres in such unpromising districts as Wales and Cumberland, the percentage of successes with inoculated seed is not significantly lower than in the south-eastern area. Taking the western and northern area as a whole it thus appears that the climatic and soil conditions do not prevent the cultivation of the crop when the nodule bacteria are supplied to it, but that the failure of the crop in these districts in the past has been mainly due to the absence

of the appropriate bacteria from the soil. It would especially appear that the Gloucestershire and Herefordshire district is well suited to the growth of lucerne from inoculated seed.

#### 7. THE CAUSES OF LUCERNE FAILURE.

The records of experiments in which the lucerne failed afford interesting evidence as to the prevalence of the various causes of failure. The trials were unfortunately started in the very wet spring and summer of 1924, and of those sown in that year 15 failed owing to weeds. The difficulty in checking the weeds in this wet year must, however, be regarded as quite abnormal. Other trials sown in this year failed for the following reasons:

Cause	No. of trials
Acid soil ... ..	3
Wet soil ... ..	2
Frost... ..	1
Too loose seed bed... ..	1
Shallow subsoil ... ..	1
Insect attack ... ..	1

The weather conditions in 1925 and 1926 were more normal. The causes of failure in trials sown in these two years were as follows:

Cause	No. of trials
Weeds ... ..	5
Drought ... ..	3
Frost... ..	3
Insect attack ... ..	1
Cause unknown ... ..	2

Even in these more normal years weeds were the most frequent form of failure. The three trials that failed through drought were sown in June or July, showing that there is some risk attending late sowing, although thirteen other trials sown in these two months have been successful. It is probable that soil acidity is a more common cause of failure than appears in these figures since in nearly all experiments the ground was well limed before the lucerne was sown. It seems clear, however, that the main difficulty to be met in extending the cultivation of inoculated lucerne in the west and north, is that of controlling weeds. There are three stages in the life of the plant when weed infestation seems to be especially dangerous. When the crop is sown in April or early May it usually has to compete in the early summer with spring germinating weeds. In the autumn of the seedling year there is often a heavy growth of such weeds as groundsel or chickweed which may smother the young lucerne. Finally, an old stand of lucerne is liable to be damaged by perennials and grasses. In many cases this last type of weed growth is probably a symptom of the weakening of the lucerne

plant from some other cause. It is in the seedling year that the real difficulty has to be met. It is necessary that the plant should be protected against severe weed competition until it is strong enough to smother the weeds, and therefore this period should be shortened as much as possible by inducing quick growth of the young plant. It is in doing this that inoculation would seem to be advantageous. It is the practice in some districts to sow the lucerne in a cover crop, and it is claimed that this helps to keep down the spring germinating weeds and also protects the young lucerne against adverse weather. On the other hand some successful growers of the crop sow it in late June or July. The advantage claimed for this method is, first that the spring germinating weeds can be removed before the lucerne is sown, and secondly that the warm soil induces rapid early growth of the crop. On the other hand the lucerne is exposed to the risk of drought in the young seedling stage and to the danger of severe competition with autumn weeds before it has made enough growth to smother them. It was thought advisable to make a comparison between these two methods of sowing the crop. Experiments were laid down in Somerset, Montgomery, Monmouth and Cumberland as well as at Rothamsted (see Table I), localities in the west being selected, since in such wet districts the difficulty of weed control is especially great. In each experiment the following treatments were tested in triplicate, the plots being arranged in three blocks within each of which the four differently treated strips were arranged at random:

- A. Inoculated seed sown in April or May in a cover crop.
- B. Untreated seed sown in April or May in a cover crop.
- C. Inoculated seed sown in July on bare ground.
- D. Untreated seed sown in July on bare ground.

In the experiment in Somerset laid down by Messrs Clarke and Sons in 1926 the cover crop failed. In the second year, however, the spring sown crop looked stronger than the July sown and was less weedy. In July 1927 weighings of the green crop were made and gave the results shown below:

Treatment	Yield in cwts. per acre. Mean of 3 plots	Percentage nitrogen in the crop
Spring sown, inoculated	119.3	4.13
Spring sown, untreated	97.2	4.09
July sown, inoculated	44.2	4.12
July sown, untreated	36.5	4.14

A similar trial laid down at the Agricultural Institute, Usk, failed probably owing to water logging of the soil in winter. In a trial laid down in 1926 at the County School, Welshpool, Montgomery, the plots became much infested with chickweed and groundsel in the autumn of

the seedling year. It was noticed that the spring sown plots were the better and the less weedy, and by the following year three of the July sown plots failed completely. In July 1927 the crop was weighed green and the following yields were obtained:

Treatment	Yield in cwt. per acre	Percentage nitrogen in the crop
Spring sown, inoculated	109.96	2.64
Spring sown, untreated	81.16	2.72
July sown, inoculated	64.2	2.39
July sown, untreated	28.8	2.50

In the trial carried out by Mr C. H. Roberts at Boothby, Brampton, Cumberland, the whole of the July sown plots as well as all the plots sown with untreated seed failed completely. Only the April sown inoculated plots bore any crop, and these produced a fair growth. The experiments agree, therefore, in showing better results from sowing the lucerne in spring with a cover crop than from July sowing. It is probable that in the west at any rate the plant needs a full summer's growth to enable it to meet the competition of autumn weeds. The comparative effects of spring and autumn sowing are no doubt greatly dependent on the weather. Trials sown in different years are therefore still required before the general superiority of the April sown crop can be established.

#### SUMMARY AND ABSTRACT.

1. The paper discusses experiments laid down at 39 centres in Great Britain to test the value of seed inoculation for lucerne.

2. The seed was inoculated by treating it with a suspension of the nodule bacteria in skim milk containing 0.1 per cent. calcium di-acid phosphate, the method developed by Thornton and Gangulee.

3. In the west and north of England the treatment greatly benefited the lucerne and often enabled a crop to be obtained where the untreated lucerne failed. At 12 centres in this area at which the crop was weighed, inoculation increased the yield by over 20 per cent. in all cases save one, where spread of the bacteria vitiated the result.

4. The improvement sometimes showed itself as an increased yield and sometimes as an increase in the nitrogen content of the hay. In most cases both these effects were produced.

5. In the midland and south central counties inoculation usually produced a temporary improvement, the untreated plant eventually catching up with the inoculated. The effect of inoculation is very much greater where the young lucerne has to compete with a cover crop. Weight results from 8 centres in this area showed increases from inoculation of over 20 per cent. in 4 cases, smaller but significant improve-

ment in yield or nitrogen content in 3 cases, and no significant effect in one case.

6. In East Anglia and Kent untreated lucerne usually develops plenty of nodules. An exceptional condition occurred in a trial at Tunstall Heath, Suffolk, on sour light land, where liming and inoculation produced a fair plant although the uninoculated lucerne developed no nodules and failed.

7. There is evidence that, when the seed is inoculated, the chances of success with lucerne are on the whole as good in the west and north of England as they are in the south-east.

8. In a number of trials sown in 1926 better results were obtained by sowing the seed in a light cover crop in spring than by sowing in June or July.

#### ACKNOWLEDGMENT.

The author wishes to thank the Royal Agricultural Society of Great Britain for the generous grants of money that have enabled the experiments above discussed to be carried out, the experimenters mentioned, besides others who kindly undertook trials in which the crop failed, and also the Agricultural officers and their assistants who supervised some of the trials and in certain cases carried out the weighing of the crops.

#### REFERENCES.

- (1) ATWATER, W. O. and WOODS, C. D. *Storrs Agric. Exp. Sta. Rept.* (1889), pp. 11-51.
- (2) THROCKMORTON, R. I. and SALMON, S. C. *Kansas Agric. Exp. Sta. Bull.* (1927), 242, pp. 5-42.
- (3) NOBBE, F. *Bot. Centr.* (1896), 68, 171-3.
- (4) DUGGAR, J. F. *Alabama Agric. Exp. Sta. Bull.* (1898), 96, pp. 183-208.
- (5) BURRILL, T. J. and HANSEN, R. *Ill. Agric. Exp. Sta. Bull.* (1917), 202, pp. 115-181.
- (6) WHITING, A. L. *J. Amer. Soc. Agronomy* (1925), 17, 474-487.
- (7) HARTLIB, S. *Legacie, or an enlargement of the Discourse of Husbandry* (1651). London: Richard Wodenoth.
- (8) WRIGHT, R. P. *West of Scot. Agric. Coll. Bull.* (1910), 53, pp. 159-170.
- (9) STEVENS, J. W. *Soil Sci.* (1925), 20, 45-62.
- (10) CUNNINGHAM, A. *Scot. J. Agric.* (1928), 11, no. 1.
- (11) SIMON, J. *Mitt. Ökon. Gesell. Sachsen* (1908), 13, 1-27.
- (12) THORNTON, H. G. and GANGULEE, N. *Proc. Roy. Soc.* (1926), Ser. B, 99, 427-451.
- (13) FRED, E. B. and DAVENPORT, A. *J. Agric. Res.* (1918), 14, 317-336.
- (14) BRYAN, O. C. *Soil Sci.* (1923), 15, 37-40.
- (15) JOFFE, J. S. *Soil Sci.* (1920), 10, 301-7.
- (16) BRYAN, O. C. *Soil Sci.* (1923), 15, 23-29.
- (17) FRED, E. B. and GRAUL, E. J. *Wisconsin Agric. Exp. Sta. Res. Bull.* (1916), 39, pp. 1-42.

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## A PRELIMINARY NOTE ON THE EFFECT OF SODIUM SILICATE IN INCREASING THE YIELD OF BARLEY.

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(With One Text-figure.)

### 1. SOURCE OF DATA.

THE permanent barley experiment at Rothamsted, of which the first crop was harvested in 1852, contained among others four plots receiving sulphate of ammonia; of these, two plots (2 and 4) received in addition 392 lb. per acre (439 Kg./Ha.) of superphosphate, while also two plots (3 and 4) received sulphates of potassium, sodium and magnesium at rates of 200, 100 and 100 lb. per acre (224, 112, 112 Kg./Ha.) respectively.

The comparison of the yields showed from the first a satisfactory response to the phosphatic manure, but little or no response to the potash and other sulphates.

For the harvest year 1864 and subsequently these four plots were each divided in two, making two series of four plots each. The first series (Series AA) continued the treatment of the previous years, while to the second series (Series AAS) a dressing of sodium silicate was added at the rate of 400 lb. per acre (448 Kg./Ha.). In 1868 the nitrogenous dressing of sulphate of ammonia was replaced by nitrate of soda at the rate of 275 lb. per acre (308 Kg./Ha.).

The remarkable effects of the addition of silicate have already attracted considerable attention, but for lack of analytical data and other reasons it appears that the effect of the addition of silicate has been in some manner misunderstood. This note presents a summary of the results of statistical analyses of the yield data, together with new chemical analyses, which appear to show conclusively that the view previously rejected that the silicate acts by making available to the plant the actual reserves of soil phosphates must be regarded as strongly established.

### 2. THE EFFECT ON THE AVERAGE CROP.

It was early realised that the plots receiving silicate were yielding considerably heavier crops than those which received no silicate, and

that this effect was especially clear on the plots which received no phosphate. Thus Hall and Morison<sup>(1)</sup> in 1906 give the following average yields in bushels per acre for the 41 years 1864–1904.

Table I. *Grain in bushels per acre.*

	Plot 1	Plot 2	Plot 3	Plot 4
Series AA (no silicate)	27.3	42.2	28.6	41.2
Series AAS (silicate)	33.8	43.5	36.4	44.5

Table II. *Straw in cwt. per acre.*

	Plot 1	Plot 2	Plot 3	Plot 4
Series AA (no silicate)	16.2	24.6	17.9	25.3
Series AAS (silicate)	19.8	25.8	21.7	27.6

In the absence of phosphate the addition of silicate has increased the yield from 28 to 35 bushels of grain, while in the presence of phosphate the increase is only from 41.6 to 44 bushels. Such results strongly suggest that the effect of the silicate, of whatever nature it may be, is intimately concerned with the phosphatic requirements of the crop. These might be either primarily a matter of soil chemistry, if the effect of the addition of silicates were to make available some portion of the phosphatic reserves of the soil, or primarily a matter of plant physiology, if its effect were to diminish the phosphatic requirements of the plant. Only analyses of the ash can settle this primary question.

It may be remarked at once, however, that the effect can scarcely be ascribed to the sodium rather than to the silicate in the manure added. For all plots receive a large quantity of sodium as nitrate, and plots 3 and 4 in addition receive further sodium, as well as potassium, as sulphate.

### 3. THE ASH ANALYSES.

Ash is available from samples of grain and straw for nearly every plot in every year. Their phosphatic content is, however, with some exceptions unknown. Hall and Morison give the phosphatic contents of the ash from grain and straw for these eight plots for the harvest years 1903 and 1904. The chemical department at Rothamsted has further supplied me this year with the values for two groups of six years each, namely 1868–73 and 1906–11. These 224 analyses thus supply data for 14 separate years.

For grain the mean content of phosphoric anhydride expressed per cent. of pure ash is shown in Table III.

Table III. *Phosphoric anhydride per cent. of ash.*

	Plot 1	Plot 2	Plot 3	Plot 4	Standard error
Series AA	31.37	35.57	30.51	35.47	0.1735
Series AAS	32.51	35.42	32.74	35.94	

The differences in percentage are comparatively small, but those induced by a dressing of superphosphate are so regular that their statistical significance can scarcely be doubted. In order to test the significance of the smaller effects it is necessary to form an estimate of the standard error of these average values. It is now becoming increasingly realised that a standard error based on the agreement of duplicate chemical determinations is not a sufficient safeguard unless such errors as arise in sampling the produce and the ash can be exhaustively examined. In order to obtain one inclusive estimate we may utilise the close parallelism between plots 1 and 2 receiving no sulphates of potash, soda and magnesium, and plots 3 and 4 which receive them. The differences between these two pairs of plots were therefore calculated for each year, and their deviations from the means of the six early and the six late years respectively provide the estimate of error given in the table, this estimate being based on 40 degrees of freedom.

Judged by this standard there is a clearly significant increase due to silicates in plots 1 and 3, but no significant change in plots 2 and 4.

In Table IV are shown the averages obtained from the analyses of the straw.

Table IV.

	Plot 1	Plot 2	Plot 3	Plot 4	Standard error
Series AA	1.997	3.598	1.830	3.533	0.0399
Series AAS	1.962	3.336	1.816	3.559	—

None of the silicate comparisons can be regarded as significant, except the somewhat large reduction of phosphatic content in the ash from plot 2. There is besides a marked increase on the plots receiving superphosphate and some decrease on those receiving potash.

The data for two years given by Hall and Morison gave indications similar to those of the average of 14 years here presented. These authors, however, write as though the ash analyses showed that phosphoric acid was less abundant in the straw (although somewhat more so in the grain), whereas the analyses in reality only show the proportion of phosphoric acid in a 100 parts of ash. They argue, therefore, that the silicate "gives the plant such a stimulus as enables it to develop more vigorously and obtain more phosphoric acid from the soil"; although on the view that

additional phosphate is not made available, it is difficult to explain why the phosphoric acid in the ash from the grain should not be somewhat decreased. The essential fact that appears to have been overlooked is that the increase of any one ingredient in the ash will *ceteris paribus* tend to diminish the percentage of other ingredients, and this effect will be most clearly apparent when we are concerned with two ingredients such as silica and phosphoric acid which contribute largely to the ash.

We may now turn to the very different picture presented by the total weight of phosphoric anhydride removed in the crop.

#### 4. WEIGHT OF PHOSPHORIC ANHYDRIDE IN THE CROP.

The total ash content of the crop, both in grain and straw, being known, it is an easy matter to calculate from the percentage of phosphoric anhydride in the ash the total content of the crop in this ingredient. This is shown with a standard error, calculated as before, in Table V.

Table V. *Phosphoric anhydride in crop (lb. per acre).*

	Plot 1	Plot 2	Plot 3	Plot 4	Standard error
Series AA	10.50	22.47	10.31	22.40	0.3873
Series AAS	13.82	22.84	15.20	25.67	—

In the absence of all phosphoric fertiliser the crops on plots 1 and 3 have depleted the reserves of phosphoric anhydride to the extent of 10.4 lb. per acre (11.7 Kg./Ha.) in each year. The effect of adding silicates to these plots has been to increase the quantity removed to 14.5 lb. per acre (16.7 Kg./Ha.), or by about 40 per cent. For comparison, the addition of superphosphate, containing, at 16.5 per cent., over 64 lb. of phosphoric anhydride per acre (72 Kg./Ha.) annually, has only increased the amount removed in the crop by 12 lb. (13.5 Kg./Ha.); and when silicate is present in addition this is further increased by nearly 2 lb. In view of these figures it is difficult to avoid the conclusion that, in the presence of silicate, phosphoric acid is made available to the plant, even in plots which have been long depleted in this ingredient without replacement, in very considerable quantities.

#### 5. THE PHOSPHATIC CONTENT OF THE CROP BY DRY WEIGHT.

The quantities of phosphoric anhydride removed in the crop, although striking in quantity and long sustained, are not competent to provide a decisive disproof of the suggestion that the effect of silicate in increasing the phosphate removed is an indirect consequence of a

stimulus to the growth of the plant. Such disproof must be sought in the phosphatic content of the crop expressed as a fraction of its dry weight. For it is evident that if the increased growth of the crop is not due to increased abundance of available phosphate but to some other nutrient or stimulus, the phosphatic content of the plant reckoned as a fraction of the total organic matter present could not be increased, but must, if any change is perceptible, be diminished; whereas, on the contrary, an increase in growth directly stimulated by an increase of available phosphates might reasonably be expected to be accompanied by an increase of phosphatic content in the organic matter. A strict application of this test would perhaps require that the phosphatic content should be reckoned per 1000 parts of dry matter *less ash*; since, however, the absolute amounts of ash, though variable, are not large, only quite negligible errors will be introduced by expressing the phosphoric anhydride in 1000 parts of dry matter.

In view of the importance of this measure we shall give the averages separately for the three periods.

Table VI. *Phosphoric anhydride per mille dry matter.*

1868-73.					Standard error
	Plot 1	Plot 2	Plot 3	Plot 4	
Series AA	3.67	4.69	3.60	4.82	—
Series AAS	4.09	4.88	4.08	5.21	
1903-4.					—
Series AA	3.46	5.36	3.08	5.19	
Series AAS	4.21	5.54	3.92	5.80	
1906-11.					—
Series AA	3.36	5.10	3.34	4.97	
Series AAS	3.85	5.25	3.80	5.20	
14 years.					0.049
Series AA	3.51	4.96	3.42	4.94	
Series AAS	4.00	5.13	3.94	5.29	

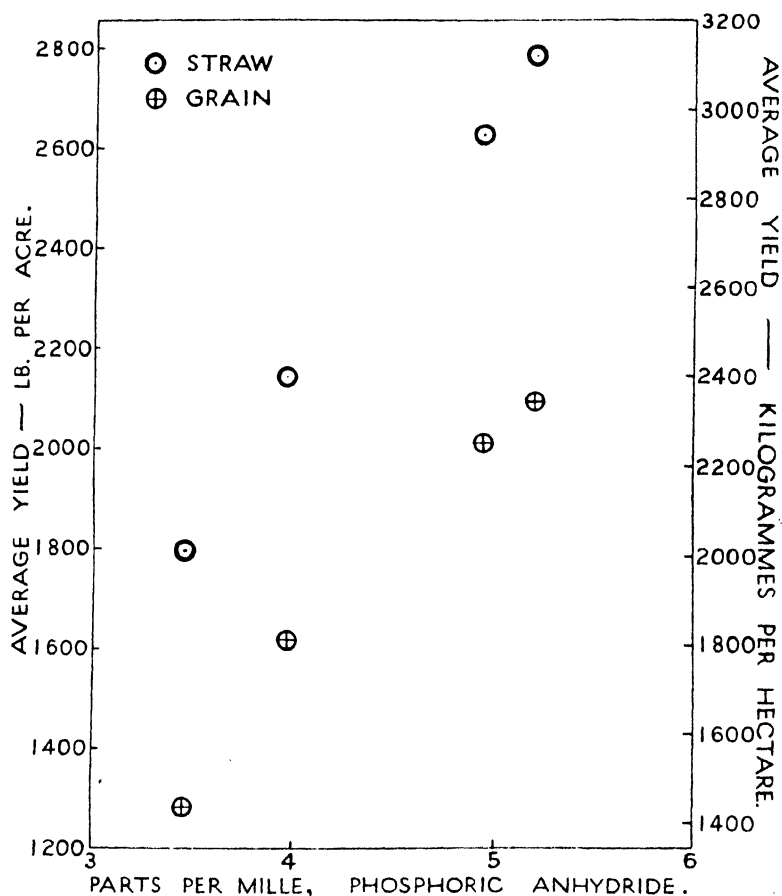
The table (Table VI) shows at all periods separately that the phosphatic content of the dry matter of the crop is increased in all four plots. It is to be noticed that the two years for which analyses are given by Hall and Morison are not the least striking in this respect. Once the results are expressed in this form, however, there can be little doubt that the addition of silicate has had at all periods, and on all the plots, the effect of making more phosphoric acid available to the crop, and not merely of stimulating growth with the secondary effect that more phosphoric acid is absorbed.

## 6. CROP INCREASE IN RELATION OF ABUNDANCE OF PHOSPHATE.

The conclusion of the last section raises the further question as to whether the crop increase associated with the addition of silicate is not

### HOOSFIELD BARLEY, (PLOTS 1-4AA & AAS)

#### RELATION BETWEEN YIELDS OF GRAIN AND STRAW AND PHOSPHORIC CONTENT OF THE CROP.



wholly accounted for by the increased abundance of available phosphate which such addition produces. We have no direct evidence of the amount of crop increase which would have been induced by smaller

additions of superphosphate than that actually employed. It is, however, reasonable to suppose that if we had the results of applying phosphate in a number of separate increments, the relation between average yield either in grain or straw and the phosphatic content of the plant would be represented by a smooth curve.

For this purpose plots 1 and 3 which differ only in the potassic fertiliser, and plots 2 and 4, may be thrown together and we obtain the results of Table VII.

Table VII.

	Phosphoric anhydride per mille dry weight	Grain. Mean yield 59 years bus. per acre	Straw. Mean yield 59 years lb. per acre
Plots 1 and 3	3.462	24.66	1794
Plots 1 S and 3 S	3.971	31.06	2144
Plots 2 and 4	4.951	38.68	2625
Plots 2 S and 4 S	5.210	40.30	2782

The values are represented graphically in the figure.

It will be seen that the values for the grain follow a very regular curve, while those for straw are somewhat less regular. Considering, however, that the standard error of our values for phosphatic content is about 0.035, it appears that neither curve can be regarded as indicating any significant departure from the simple view that the whole of the increased yield both in grain and straw associated with the dressing of sodium silicate is solely ascribable to the increased availability of the phosphatic reserves of the soil. If the results can at all be expressed in terms of stimulus, it is a stimulus to phosphatic intake only, and not to plant growth, that must be postulated.

#### SUMMARY.

The addition of sodium silicate has been found to increase the yield of barley to a considerable extent, this effect being most marked when no superphosphate is added.

The phosphatic content of the ash is not greatly increased in the grain, and is diminished in one case in the straw; the conclusion from this observation that the silicate does not act by releasing soil phosphates, but as a plant stimulus, overlooks the fact that the addition of silica to the ash naturally reduces the percentage of other constituents, and should be discounted.

The phosphate removed annually in the crop is greatly increased on the plots receiving silicate, even when this removal has continued for many years without replacement.

That additional phosphate is actually made available to the crop on the plots receiving silicate is shown by the increase in the proportion of phosphate in the dry weight of the crop, which appears on all the plots, and at all periods.

This increase is quantitatively sufficient to account for the increased yield in grain and straw, without postulating the aid of any stimulus to plant growth.

#### REFERENCE.

- (1) HALL, A. D. and MORISON, C. G. T. On the Function of Silica in the Nutrition of Cereals. *Proc. Roy. Soc.* (1906), B, 77, 455-477. (Rothamsted Memoirs, vol. VIII.)

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# STUDIES IN CROP VARIATION.

## V. THE RELATION BETWEEN YIELD AND SOIL NUTRIENTS

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### 1. INTRODUCTION.

IN spite of the manifest progress of plant physiology a deductive theory of the relation between the size of a plant and the environmental factors seems still remote. Our present knowledge is too small and scattered to serve as a basis for the formulation of any rigid laws. The only procedure open therefore to quantitative research is to attempt to establish empirically some relationship between yield and external factors; it may thereafter be possible to discover its physiological significance, and in any case to put it to practical use.

Liebig made an early attempt to formulate a relationship between the plant-yield and the intensity of the external factors. He stated that of all the factors there was one which was "in minimum" and that the size of the crop was determined by, and increased with, increase in this factor until a point was reached at which some other factor was in minimum. Mitscherlich and many other workers have, however, shown conclusively that the simple formula of Liebig is not a true expression of the yield-factor relationship. The law of plant-yield formulated by Mitscherlich (1), his expression for the relation between the yield and the environmental factors and the practical use and physiological significance of this expression, have all been very severely criticised by

Briggs<sup>(2)</sup>. Throughout his work Mitscherlich stresses his idea that the value of the constant  $c$  in his expression

$$y = A(1 - e^{-cx}),$$

expressing the yield  $y$  in terms of the quantity of nutrient  $x$ , is dependent upon the nature of the factor and upon that alone, and yet it is precisely the change in the relation of growth to the single factor brought about by the interaction of other factors that needs to be known. In fact Briggs has successfully shown from Mitscherlich's own data that where only one factor, such as potassium, is varied at a time the relation between yield and factor is different for different values of the other nutrient—magnesium. In most cases where manure is added in the form of a salt, the factors increase simultaneously. When two factors with different constants are increased simultaneously the value of  $dy/dx$  is no longer given by the simple relation  $dy/dx = c(A - y)$ , and is thus no longer proportional to the deficit from the maximum. The relations worked out for two factors by Briggs show that if in investigating the effect of a single substance, such as potassium, a salt such as phosphate or nitrate is added, the conditions should be such that sufficient of the elements introduced as part of the salt (phosphate or nitrogen) is already present to render the effect of further increase of these elements negligible. More detailed data is surely required to decide the point raised by Mitscherlich that the constants for nitrogen are the same in the form of ammonium, urea and nitrate ions. Mitscherlich's work has paved the way for further thorough investigation, but his results bring out only two tangible facts, (i) that yield will be increased by an increase in any of the factors, provided  $c$  is positive, and (ii) that the value of  $dy/dx$  is proportional to the corresponding factor  $c$  and to the deficit of the yield at that point from the maximum attained when that particular factor is increased indefinitely. The hypothesis that "as far as variations in one factor alone are concerned the yield expressed as a fraction of the maximum yield obtained when that factor is very great is the same whatever may be the plant, portion of plant, time of harvest, and other growth conditions in weather, soil, etc." is not only open to challenge but entirely belied by agricultural as well as pot-culture data (see section 2). Mitscherlich has attempted to use his equation in conjunction with the results of pot-culture experiments to determine (a) the amount of a manurial substance in a sample of soil, and (b) the effect of the addition of artificial manure to field plots. In this practical use of the equation he does not employ any well recognised statistical method, and

with the adaptability of  $A$  and  $b$  allowed by him it is not surprising that values for the constants are found so that the values of  $y$  calculated on their basis give an agreement with observation which Mitscherlich considers satisfactory, although the main justification brought forward by him for his results is the agreement of the observed and the calculated values. Mitscherlich makes no attempt to measure statistically the precision of values obtained for his constants. In the absence of their standard errors it is difficult to judge the goodness of fit of these values, and their practical applications also become a matter of great doubt. All the evidence considered by Mitscherlich is based upon pot cultures and he himself seems to be in great difficulty to apply it in a suitable manner to field conditions.

## 2. STATEMENT OF THE PROBLEM.

Briggs has clearly shown that even in approaching the subject from Mitscherlich's direction we are not limited to his narrow path. From our knowledge of plants it is unlikely that when the conditions are made more and more favourable the size of a plant can go on increasing without limit. There are various possible yield-factor relations. The law of diminishing returns may hold throughout the whole range; the yield may increase proportionately with increased quantity of manure up to a certain point and then no further as Liebig suggested, although not independently of the interaction of other factors (a fact which was lost sight of by him); the return per outlay may increase with increase in outlay up to a certain point and then no further increase in the return will result; or we may have various combinations of these relations, but beyond a maximum return we cannot go. In the case of the plant and variation in one factor of the environment the law of diminishing returns seems to apply. The Mitscherlich one-factor equation is not the only equation which approaches a maximum value with ever decreasing return per outlay as outlay is increased. In fact Briggs has shown that some other equations fit even Mitscherlich's data much more closely than his particular equation. More recently Lemmerman (3) has come to the same conclusion. However, it is necessary to the understanding of the problem to examine the more general implications of Mitscherlich's yield equation  $y = A(1 - e^{-cx})$ . If the intensity of the factor changes to  $x'$ , the value of the yield  $y'$  will equal the value  $A(1 - e^{-cx'})$ , or the change of  $y$  to  $y'$  will take place in the ratio  $(1 - e^{-cx}) : (1 - e^{-cx'})$ , i.e. independently of the value of  $A$ . All other factors governing the yield, besides the one under consideration, can affect the yield only by pro-

ducing some change in the value of  $A$ ; this ratio, being independent of  $A$ , is therefore required to be uninfluenced by the condition of other factors at the time of the change. That such ratios are not in fact independent of the interaction of other factors can be shown from any fairly accurate agricultural data. The well-known data on wheat-yield from Broadbalk(4) supply a very clear case; the addition of a certain dose of nitrogen raises the yield in the ratio 1.589 : 1 (plots 3, 4 and 10) when no other salts are added, but the same dose of nitrogen in the presence of mineral salts is capable of raising the yield in the ratio 2.212 : 1 (plots 5 and 7).

Table I (A). *Broadbalk data. Manure per acre, in lb.*

Plot	Sul-phate of potash	Sul-phate of soda	Sul-phate of mag-nesia	Super-phosphate	Sul-phate of ammonia	Chloride of ammonia	Mean bushels per acre	$y'$ $y$	$\frac{1}{y} - \frac{1}{y'}$
3 and 4	—	—	—	—	—	—	$y$ 12.269	1.589	.03021
10	—	—	—	—	200	200	$y'$ 19.504		
5	200	100	100	392	—	—	$y$ 14.180	2.212	.03765
7	200	100	100	392	200	200	$y'$ 31.367		

The same fact is clearly borne out by the yields of barley taken from the pot-culture investigations of Dr Gregory at Rothamsted regarding the interaction of nitrogen and phosphorus. Here the ratio of the yield obtained when 1215 mg. of nitrogen per pot are supplied to that when only 15 mg. are given, varies from 4.902 to 24.000 as the amount of phosphorus added rises from 5 to 405 mg. per pot.

Table I (B). *Pot-culture data.*

$P_2O_5$	Mg. of nitrogen per pot		Ratio of yields	Reciprocal difference
	15	1215		
5	3.06	15.00	4.902	.2602
15	3.36	17.88	5.321	.2417
45	3.03	29.60	9.769	.2963
135	2.39	68.10	20.089	.2784
405	3.60	86.40	24.000	.2663

Mitscherlich's yield-factor relation fits neither the agricultural nor the pot-culture data, and it therefore fails to meet a requirement of yield formulae, which has been far too little appreciated. Not only must the formula represent satisfactorily the response to variations of a single factor, but also the response to simultaneous variations of two or more different factors. A yield-factor relation suggested by Maskell is critically tested in this paper and seems to fulfil this necessity. This, which by electrical analogy may be termed the Resistance Formula, requires that, instead of the ratio  $y'/y$  being independent of the influence of other factors,

when the value of one factor changes in intensity,  $\frac{1}{y} - \frac{1}{y'}$ , the difference of reciprocals of yields, should behave as such; the agreement of the several values of this difference both in the case of the field data and in that of the pot-culture data suggests that we have here at least a good approximation.

The succeeding sections are devoted to testing, by comparison with the best available data, formulae of the general resistance type, *i.e.*

$$\frac{1}{y} = F(N) + F'(K) + F''(P) + \dots + C,$$

and the derived Special Formulae in which  $F, F', F'' \dots$  are inversely proportional to the nutrients available.

The purpose of this paper is therefore to show:

(a) By a method of approximation, detailed hereinafter, the satisfactory fit of the Resistance Formula to several sets of data.

(b) That the expressions  $F(N)$  and  $F'(K)$ , etc. are well represented by the forms  $\frac{a_n}{n + N}$  and  $\frac{a_k}{k + K}$ , etc., where  $N$  and  $K$ , etc. are the nutrients added to the soil and  $n, k$ , etc. those originally present in the soil, while  $a_n, a_k$ , etc. are the constants for the several types of manure added.

(c) That the harvesting data, when fairly freed of experimental errors by the method of approximation shown in section 3 (a), can be used for evaluating the terms  $a_n, a_k, n$  and  $k$ , and that these parameters obtained satisfactorily fit the experimental data.

(d) That the values of  $n$  and  $k$  obtained through this channel give us a new measure of soil fertility, which differs from the absolute quantity of plant food present in the soil in that it is rather the measure of the effect of the plant food in the soil in terms of the soil fertiliser. This new measure of soil fertility affords direct and independent evidence necessary to test the validity of laboratory methods for estimating the amount of the several nutrients in the soil. These values should also enable us to measure the immediate availability of plant food in different manures and their residual value for the succeeding crops.

(e) That the standard errors of the values of the several terms obtained can be measured. This is necessary to obtain trustworthy conclusions free from any subjective bias. These are also required in the design of agricultural experiments capable of yielding more precise and accurate results.

(f) That these values of the constants when obtained with sufficient accuracy should enable us to answer such problems as the "optimum value for a given manure" or a balanced manure for two or more kinds of nutrients with great confidence.

### 3. MATERIAL AND METHODS OF INVESTIGATION.

#### (a) *General Resistance Formula.*

The choice of material for the development of the method was due solely to circumstances. The absence of any very accurate data seems to stand for the present in the way of the investigations being pushed much farther beyond the present stage. The writer is, however, of opinion that not only is the method employed essentially applicable to field trials and pot cultures, but that it could be applied to any type of plant-yield data by introducing suitable modifications in the system of weighting, etc. The following example is chosen to illustrate the process of fitting and testing the Resistance Formula.

Table II. *Potatoes (Kerr's Pink), Stackyard Field, Rothamsted, 1926.*  
*Average yield in tons per acre (y).*

		Cwt. per acre, sulphate of potash				
		0	1	2	4	
Cwt. per	0	7.80	7.80	8.01	7.79	System of replication: Randomised blocks for all manurial combinations. Plots 1/50 acre. Basal dressing 3 cwt. superphosphate per acre.
acre, sul-	1	7.73	8.98	9.17	9.01	
phate of	2	9.40	10.56	10.30	10.44	
ammonia	4	9.53	11.15	11.62	12.34	

*Approximation.* As already stated the central idea in the Resistance Formula, is that the reciprocal of the yield  $\left(\frac{1}{y}\right)$  is the sum of several portions, each a function of one nutrient, like  $F(N)$ ,  $F'(K)$ , etc., and that fact is here utilised in building up a series of expectations of yield. The measure of agreement of the expected yields with the observed yields would thus afford us a means to judge the accuracy of our hypothesis.

It is first necessary to obtain crude values of the expected yields, that is, values obeying the Resistance Formula and approximate to those observed. The fourth powers of these crude expectations may then be used as weights in obtaining the improved fit.

Table III. *Reciprocals of the yield*  $\left(\frac{1}{y} \text{ or } x\right)$ .

				Means of rows (a)
	·1282	·1282	·1248	·1284
	·1294	·1114	·1090	·1110
	·1064	·09470	·09709	·09579
	·1049	·08969	·08606	·08104
Means of columns (b)	·1172	·1060	·1042	·1041
				·1079 (c)

Table IV (A). *Reciprocals of expected yields*  $\left(\frac{1}{m}\right)$  *built from the margins of Table III by the summation formula*  $a + b - c$ .

·1367	·1255	·1237	·1236
·1245	·1133	·1115	·1114
·1078	·09659	·09479	·09469
·09972	·08852	·08672	·08662

Table IV (B). *Values of*  $m$ .

7·315	7·968	8·084	8·091
8·032	8·826	8·969	8·977
9·276	10·353	10·550	10·561
10·028	11·297	11·531	11·545

$$\Sigma (y - m)^2 = 1·5331.$$

Table IV (c). *Values of*  $m^2$ .

				Sums of rows
2,863	4,031	4,271	4,286	15,451
4,162	6,068	6,471	6,494	23,195
7,404	11,489	12,388	12,440	43,721
10,112	16,287	17,679	17,761	61,839
Sums of columns	24,541	37,875	40,809	40,981
				144,206

The margins in the next table (Table V) are built up by taking the weighted averages of the reciprocals in Table III row by row and column by column. The weights are taken equal to the corresponding values of  $m^2$ , these being inversely proportional to the variances of the reciprocals in field trials, where the variance in yield is usually independent of yield. This set of weights makes the residual variance approximately a minimum. In the example from pot cultures (see Appendix II) where the variance increases with the value of the yield, the total variance becomes least when the weights are taken equal to the corresponding values of  $m^2$ . The value ·09958 (Table V) is the weighted average of the 16 entries of Table III, and it provides a useful arithmetical check to obtain it from each set of marginal means in turn. This value ·09958 has been subtracted

from the row margins to facilitate the arithmetic. The several values of  $\left(\frac{1}{m}\right)$  in Table V are then obtained by adding the marginal values  $p_s$  and  $q_t$ .

Table V. *Reciprocals of expectations*  $\left(\frac{1}{m}\right)$ .

				$q_t$		
	·13993	·12652	·12483	·12295	·12731	·02773
	·12646	·11305	·11136	·10948	·11384	·01426
	·11029	·09688	·09519	·09331	·09767	− ·00191
	·10127	·08786	·08617	·08429	·08865	− ·01093
$p_s$	·11200	·09879	·09710	·09522	·09958	

The values of  $m$  are calculated and thence the sum of the squares of the residuals  $\Sigma (y - m)^2$  which is found to be 1·294. The drop in the value of  $\Sigma (y - m)^2$  from the previous figure 1·5331 to 1·294 shows that the fit of the expected yields ( $m$ ) to the observed values ( $y$ ) has considerably improved by this process of weighting.

The approximate values of the margins  $p_s$  and  $q_t$  are then improved with a view to making the sum of products of the weights and expectations equal to the sum of products of the weights and the actual yields along rows and columns. The several corrections required are calculated as follows:

Let  $a_{11}, a_{12}, a_{21}, a_{22} \dots a_{44}$  be the true reciprocals of expectations,  $x_{11}, x_{12}, x_{21}, x_{22} \dots x_{44}$  the reciprocals of the observed yields, and

$$w_{11}, w_{12}, w_{21}, w_{22} \dots x_{44}$$

the weights as used above; then

$$w_{11}a_{11} + w_{12}a_{12} + w_{13}a_{13} + w_{14}a_{14} = w_{11}x_{11} + w_{12}x_{12} + w_{13}x_{13} + w_{14}x_{14}$$

.....

$$w_{41}a_{41} + w_{42}a_{42} + w_{43}a_{43} + w_{44}a_{44} = w_{41}x_{41} + w_{42}x_{42} + w_{43}x_{43} + w_{44}x_{44}$$

along columns,

and  $w_{11}a_{11} + w_{21}a_{21} + w_{31}a_{31} + w_{41}a_{41} = w_{11}x_{11} + w_{21}x_{21} + w_{31}x_{31} + w_{41}x_{41}$

.....

$$w_{14}a_{14} + w_{24}a_{24} + w_{34}a_{34} + w_{44}a_{44} = w_{14}x_{14} + w_{24}x_{24} + w_{34}x_{34} + w_{44}x_{44}$$

along rows.

Suppose  $a_{st} = p_s + q_t$  where  $p_s$  and  $q_t$  represent true marginal values; then by substitution we get the equations:

$$(w_{11} + w_{12} + w_{13} + w_{14}) p_1 + w_{11}q_1 + w_{12}q_2 + w_{13}q_3 + w_{14}q_4$$

$$= S(w_{1t}x_{1t}), \text{ etc. along columns (4 equations) } \dots\dots\text{(I),}$$

and similarly

$$(w_{11} + w_{21} + w_{31} + w_{41}) q_1 + w_{11}p_1 + w_{21}p_2 + w_{31}p_3 + w_{41}p_4$$

$$= S(w_{s1}x_{s1}), \text{ etc. along rows (4 equations) } \dots\dots\text{(II),}$$

or 
$$p_1 = \frac{S(w_{1t}x_{1t})}{S(w_{1t})} - \frac{S(w_{1t}q_t)}{S(w_{1t})} \dots\dots(\text{equations I}),$$

and 
$$q_1 = \frac{S(w_{s1}x_{s1})}{S(w_{s1})} - \frac{S(w_{s1}p_s)}{S(w_{s1})} \dots\dots(\text{equations II}).$$

By inserting in equations (I) the values of  $q$  in the last table, we obtain a new series of  $p$ . Then from this new series of  $p$  we re-calculate the values of  $q$  by equations II.

The corrected values of  $p$  and  $q$  are now used to build a new table (VI) of reciprocals of expectations:

Table VI. *Table of reciprocals of expectations*  $\left(\frac{1}{m}\right)$ .

				$q_t$	
	·13913	·12633	·12475	·12287	·02750
	·12575	·11295	·11137	·10949	·01412
	·10973	·09693	·09535	·09347	— ·00190
	·10080	·08800	·08642	·08454	— ·01083
$p_s$	·11163	·09883	·09725	·09537	

A table of weighted discrepancies between the observed and the expected values is then prepared by multiplying the differences  $\left(\frac{1}{y} - \frac{1}{m}\right)$  by the weights used above. If the discrepancies are well distributed, the marginal totals ought to be very small compared to the individual values in the corresponding rows or columns as in Table VII. If however it is not so, the margins are further corrected as shown below and a similar table again calculated, when the condition for the fair distribution of discrepancies will be found to be more nearly fulfilled.

Table VII. *Weighted discrepancies between reciprocals of observed and expected values of yield.*

- 31·293	+ 7·538	+ 0·214	+ 23·702	+ ·161
+ 15·191	- 9·405	- 15·336	+ 9·806	+ ·256
- 24·655	- 25·620	+ 21·555	+ 28·861	+ ·141
+ 41·459	+ 27·525	- 6·364	- 62·164	+ ·456
+ 0·702	+ 0·038	+ 0·069	+ 0·205	1·014

Suppose  $e_1, e_2, e_3, e_4$  and  $f_1, f_2, f_3, f_4$  are the corrections required in the values of  $p$  and  $q$  as determined from equations (I) and (II) in order to bring them as near the true expectations as possible. Then equations (I) can be written as

$$(w_{11} + w_{12} + w_{13} + w_{14})(p_1 + e_1) + w_{11}(q_1 + f_1) + w_{12}(q_2 + f_2) + w_{13}(q_3 + f_3) + w_{14}(q_4 + f_4) = S(w_{1t}x_{1t}) - \cdot 702,$$

or 
$$S(w_{1t}) \cdot e_1 + w_{11}f_1 + w_{12}f_2 + w_{13}f_3 + w_{14}f_4 = - \cdot 702.$$

Assuming the set of margins  $q_i$  to be correct, the values of  $f$  are taken to be zero, and in that case

$$e_1 = \frac{-.702}{S(w_{1t})} \text{ and so on.}$$

Similarly the equations (II) give

$$S(w_{s1}) \cdot f_1 + w_{11}e_1 + w_{21}e_2 + w_{31}e_3 + w_{41}e_4 = -.161, \text{ etc.,}$$

and the values of  $f$  are calculated from the values of  $e$  now determined. The values of the quantities  $e$  and  $f$  for the case in hand were found to be as follows:

$$\begin{array}{cc|cc} e_1 & = .00003 & f_1 & 0 \\ e_2 & 0 & f_2 & 0 \\ e_3 & 0 & f_3 & 0 \\ e_4 & = .00001 & f_4 & 0. \end{array}$$

Applying these corrections, the new values for  $p$  and  $q$  (now denoted by  $p_i$  and  $q_i$ ) were obtained and therefrom tables for the reciprocals of expectations, the expectations and the weighted discrepancies between the observations and the expectations were calculated.

Table VIII (A). *Reciprocals of expectations*  $\left(\frac{1}{m}\right)$ .

				$q_i$
	.13916	.12633	.12475	.12288
	.12578	.11295	.11137	.10950
	.10976	.09633	.09535	.09348
	.10083	.08800	.08642	.08455
$p_i$	.11166	.09883	.09725	.09538

Table VIII (B). *Expectations* ( $m$ ).

7.184	7.918	8.013	8.137
7.949	8.850	8.977	9.132
9.107	10.317	10.488	10.697
9.921	11.364	11.571	11.827

$$\Sigma (y - m)^2 = 1.341.$$

Table VIII (C). *Weighted discrepancies*  $\left[w \left(\frac{1}{y} - \frac{1}{m}\right)\right]$ .

- 31.378	+ 7.538	+ .214	+ 23.659	+ .033
+ 15.066	- 9.405	- 15.336	+ 9.741	+ .066
- 24.877	- 25.620	+ 21.555	+ 28.736	- .206
+ 41.156	+ 27.525	- 6.364	- 62.341	- .024
- .033	+ .038	+ .069	- .205	- .131

In case the marginal values of the weighted discrepancies in Table VIII (C) are still found to be large, the fourth powers of the values of  $m$  in Table VIII (B) are taken as improved weights and the whole process repeated. This was found necessary in the case of the pot-culture data.

(b) *Special Resistance Formula.*

It has been shown in the previous section that the reciprocal of the yield  $\frac{1}{y}$  can be built up by adding the two expressions  $F(N)$  and  $F'(K)$ . This section deals with the investigation of the approximate forms of these expressions. It appears from the analysis made in the succeeding pages that  $F(N)$  and  $F'(K)$  are within a sufficient degree of accuracy of the forms  $\frac{a_n}{n + N}$  and  $\frac{a_k}{k + K}$ .  $a_n$  and  $a_k$  vary with the nutrient and the crop and are therefore called importance factors: for they measure the importance of the particular nutrient to the crop manured. Possibly they would vary to some extent with the nature of the soil and weather conditions, but such variations are not evident in the data used for the present paper.  $k$  and  $n$  represent the available potash and nitrogen in the unmanured soil in terms of cwt. of sulphate of potash and sulphate of ammonia per acre. The values for these constants were determined as shown below.

From special Resistance Formula:

$$p_1 = c + \frac{a_k}{k + K_1} \quad \text{and} \quad p_4 = c + \frac{a_k}{k + K_4},$$

$$\therefore p_1 - p_4 = a_k \frac{K_4 - K_1}{(k + K_1)(k + K_4)} \quad \text{or} \quad \frac{K_4 - K_1}{(p_1 - p_4)} = (k + K_1) \frac{k + K_4}{a_k}$$

.....(III),

and from the values of  $p_i$  given in Table VIII (A) we get:

Table VIII (D).

Manure ( $K_i$ ) added in cwt. per acre	1 Total manure ( $k + K_i$ )	2 $p_i$	3 $p_i - p_4$	4 $\frac{K_4 - K_i}{p_i - p_4}$
0	$K + 0$	.11166	.01628	245.7
1	$K + 1$	.09883	.00345	869.6
2	$K + 2$	.09725	.00187	1069.5
4	$K + 4$	.09538		

We expect from the reasoning given in equation (III) that the values given in columns 1 and 4 of the above table when plotted ought to give a straight line graph. The point where this line cuts the  $x$ -axis gives us roughly a measure of  $k$ , the potash manure available in the unmanured soil. The value of  $k$  is found to be equivalent to an added manure of 0.8 cwt. of sulphate of potash per acre. From this approximate value of  $k$  the value of  $a_k$  was calculated to be .0156. These values of  $k$  and

$a_k$  were used to prepare the next table (VIII (E)), the value of  $c$  in each case being obtained by subtracting the values in column 3 from those in column 2.

Table VIII (E).

1	2	3	4	5	6
$k + K_t$	$p_t$	$a_k$ $k + K_t$	$c$	$c + \frac{a_k}{k + K_t}$ ( $c = .09100$ approximately)	$c + \frac{a_k}{k + K_t} - p_t = \bar{r}_t$
.8	.11166	.01950	.09216	.11050	-.00116
1.8	.09883	.00866	.09017	.09966	+.00083
2.8	.09725	.00557	.09168	.09657	-.00068
4.8	.09538	.00411	.09127	.09511	-.00027

Assuming the approximate value of  $c$  to be .09100, the values in column 5 were obtained by adding this value to the corresponding values of

$\frac{a_k}{k + K_t}$  in column 3.

The next problem is to fit the values in column 5 to the corresponding values obtained from the General Formula and given in column 2. The necessary corrections in the values of the quantities  $c$ ,  $a_k$  and  $k$  for the purposes of best fit were calculated by the method of least squares as follows:

Let  $Q = \sum_{t=1}^4 \left\{ w_t \left( c + \frac{a_k}{k + K_t} - p_t \right)^2 \right\}$  be the expression to be minimised, and  $a_k$ ,  $k$  and  $c$  be an approximate solution; then the next approximation  $a + a''$ ,  $k + k''$ ,  $c + c''$  will be given by the equations

$$0 = \frac{\partial Q}{\partial (a_k + a'')} = \frac{\partial Q}{\partial a_k} + a'' \frac{\partial^2 Q}{\partial a_k^2} + k'' \frac{\partial^2 Q}{\partial a_k \partial k} + c'' \frac{\partial^2 Q}{\partial a_k \partial c},$$

$$0 = \frac{\partial Q}{\partial (k + k'')} = \frac{\partial Q}{\partial k} + a'' \frac{\partial^2 Q}{\partial a_k \partial k} + k'' \frac{\partial^2 Q}{\partial k^2} + c'' \frac{\partial^2 Q}{\partial k \partial c},$$

$$0 = \frac{\partial Q}{\partial (c + c'')} = \frac{\partial Q}{\partial c} + a'' \frac{\partial^2 Q}{\partial a_k \partial c} + k'' \frac{\partial^2 Q}{\partial k \partial c} + c'' \frac{\partial^2 Q}{\partial c^2},$$

where

$$\frac{\partial Q}{\partial a_k} = \sum_{t=1}^4 \frac{2w_t}{k + K_t} \left( c + \frac{a_k}{k + K_t} - p_t \right), \quad \frac{\partial^2 Q}{\partial a_k^2} = \sum_{t=1}^4 \frac{2w_t}{(k + K_t)^2},$$

$$\frac{\partial Q}{\partial k} = - \sum_{t=1}^4 \frac{2w_t a_k}{(k + K_t)^2} \left( c + \frac{a_k}{k + K_t} - p_t \right),$$

$$\frac{\partial^2 Q}{\partial k^2} = \sum_{t=1}^4 \left\{ \frac{2w_t a_k^2}{(k + K_t)^4} + \frac{w_t a_k}{(k + K_t)^3} \left( c + \frac{a_k}{k + K_t} - p \right) \right\},$$

$$\begin{aligned}\frac{\partial Q}{\partial c} &= \sum_{t=1}^4 2w_t \left( c + \frac{a_k}{k + K_t} - p_t \right), \quad \frac{\partial^2 Q}{\partial c^2} = \sum_{t=1}^4 (2w_t), \\ \frac{\partial^2 Q}{\partial a_k \partial k} &= - \sum_{t=1}^4 \frac{2w_t a_k}{(k + K_t)^3} + \frac{2w_t}{(k + K_t)^2} \left( c + \frac{a_k}{k + K_t} - p_t \right), \\ \frac{\partial^2 Q}{\partial a_k \partial c} &= \sum_{t=1}^4 \frac{2w_t}{k + K_t} \quad \text{and} \quad \frac{\partial^2 Q}{\partial c \partial k} = - \sum_{t=1}^4 \frac{2w_t a}{(k + K_t)^2}.\end{aligned}$$

The evaluation of the quantities  $\frac{\partial Q}{\partial a_k}$ ,  $\frac{\partial^2 Q}{\partial a_k^2}$  and  $\frac{\partial^2 Q}{\partial a_k \partial k}$ , etc. is facilitated by the preparation of a table like the following (VIII (r)):

Table VIII (r).

$\delta_t$	$w_t$	$k + K_t$	$\frac{w_t}{k + K_t}$	$\frac{w_t}{(k + K_t)^2}$	$\frac{w_t}{(k + K_t)^3}$	$\frac{w_t}{(k + K_t)^4}$
-·00116	24,541	·8	30,676	38,345	47,931	59,913
+·00083	37,875	1·8	21,042	11,690	6,494	3,608
-·00068	40,809	2·8	14,575	5,205	1,859	664
-·00027	40,981	4·8	8,538	1,779	371	77
			74,831	57,019	56,655	64,262

The values of  $\frac{\partial Q}{\partial a_k}$ ,  $\frac{\partial^2 Q}{\partial a_k^2}$ ,  $\frac{\partial^2 Q}{\partial a_k \partial k}$ , etc. are then substituted in the equations derived from minimising the sum of the squares of the differences and the equations solved for  $a''$ ,  $k''$  and  $c''$ . The corrections  $a''$ ,  $k''$  and  $c''$  were found to be -·0067, -·32 and -·00179 respectively. The corrected values of  $a_k$ ,  $k$  and  $c$  therefore become ·0089, ·48 and

·09279, and these are used to calculate the new values of  $p$ , i.e.  $c + \frac{a_k}{k + K_t}$

best fitted with the values of  $p$  obtained from the General Formula. By an exactly similar process the values of the quantities  $a_n$ ,  $n$  and  $c'$  were determined from the values of  $q$  in Table VIII (A) and found to be ·0986, 1·7275 and -·02771. The new values of  $q$  were then calculated from the relation  $q_t = c' + \frac{a_n}{n + N_t}$ . These values of  $p$  and  $q$  were then used for building up reciprocals of expectations obeying the implications underlying the special Resistance Formula. These expectations are given in Table IX.

Table IX (A). *Reciprocals of expectations from special**Resistance Formula*  $\left(\frac{1}{m}\right)$ .

				$q_i$
·14070	·12817	·12575	·12415	·02937
·11977	·10724	·10482	·10322	·00844
·11007	·09754	·09512	·09352	— ·00126
·10084	·08831	·08589	·08429	— ·01049
$p_i$ ·11133	·09880	·09638	·09478	

Table IX (B). *Expectations from special Resistance Formula*  $(m)$ .

7·10	7·80	7·95	8·05
8·35	9·33	9·54	9·69
9·08	10·25	10·51	10·69
9·92	11·32	11·64	11·86

$$\Sigma (y - m)^2 = 2.3832.$$

(c) *Other data examined.*

Two more sets of data on potato crops were next examined. These were made available by the courtesy of the authorities of the Seale-Hayne Agricultural College. These two crops were sown and raised under almost the same conditions with the only difference that in the case of one the plots were uniformly manured with 10 tons of dung per acre, whereas in the other crop the plots were left undunged. Otherwise in both cases four plots were devoted to each treatment as in the experiment on Stackyard Field discussed in section 3 (a), and the doses of the sulphate of ammonia and sulphate of potash were 0, 1, 2 and 3 cwt. per acre. A close examination of the values of constants determined from these two sets of data reveals many points of great interest. Whereas in the undunged plots the addition of the potash manure had a slightly depressing effect ( $a_k = -\cdot0075$ ), if any, the addition of dung made the same soil in a position to profit nearly three times ( $a_k = \cdot0258$ ) as much as in the case of Stackyard Field ( $a_k = \cdot0089$ ). Similarly the importance of nitrogen to the crop when the Seale-Hayne soil was undunged ( $a_n = \cdot0788$ ) was slightly less than that in the case of Stackyard Field, but with the dung added to the soil this importance of nitrogen ( $a_n = \cdot168$ ) became nearly twice as much. The farmyard manure thus not only enriches the soil in the way of adding to it definite quantities of nitrogen and potash, but also changes the physical condition of the soil and makes it more susceptible to the advantages of adding further doses of artificial fertilisers. The differences between the values of  $n$  and those of  $k$  obtained from these two sets of data give us a measure of the nitrogen and potash made available to the plant by the addition of farmyard manure. This point is more fully dealt with in subsection (e).

Besides these sets of data in potato crops the yields of barley pot cultures grown by Dr Gregory at Rothamsted have been examined and the results embodied in Appendix II. This experiment was conducted to study the reaction of nitrogen and phosphatic manures. In the case of nitrogen 15, 45, 135, 405, 1215 mg. were added per pot, whereas in the case of  $P_2O_5$  the doses were 5, 15, 45, 135, 405 mg. per pot. The whole arrangement resulted in 25 different treatments. The value of  $a_n$  is nearly eight times the value of  $a_p$ , showing the comparatively much greater importance of nitrogen in increasing the yields. Although the sand used for growing the cultures is washed free of nitrogen it is found to have available to the plant nitrogenous food equivalent to 13.947 mg. of nitrogen added in the form of the fertiliser. The source of this nitrogen and its value have been critically examined in subsection (c). That the sand is found to contain available plant-food equivalent to 16.4 mg. of  $P_2O_5$  does not require any explanation.

(d) *Tests of goodness of fit.*

It was shown in section 2 that the natural sequence of Mitscherlich's one-factor relation is to expect that the proportionate change in yield produced by varying the intensity of one factor is not influenced by the prevailing intensities of the other factors, and that this expectation is belied by both field experiments as well as pot cultures. Let us now examine the similar sequence of the Resistance Formula with a view to testing its agreement with the several data handled. The one-factor relation as obtained from the Resistance Formula is  $\frac{1}{y} = c + \frac{a_n}{n + N}$ . When the intensity of  $N$  changes to  $N'$ , the corresponding yield  $y'$  is given by the relation  $\frac{1}{y'} = c + \frac{a_n}{n + N'}$ . The difference between the reciprocals of the two yields, *i.e.*  $\frac{1}{y} - \frac{1}{y'}$ , is independent of  $c$  or the influence of the other nutrients or prevailing conditions of the experiments. That this expectation is borne out by the experimental data will be seen from a perusal of the values of  $\frac{1}{y} - \frac{1}{y'}$  obtained in Tables I (A) and (B). It can be easily shown that this relation leads us to expect that the yields will increase with the increase in the quantity of the manure applied and that this rate of increase of yield falls down as the intensity of the manurial application becomes greater and greater.

*Fit of the General and Special Formulae.*

The precise fit of these two formulae was however tested by the method of the analysis of variance as developed by Dr R. A. Fisher(5). In the Rothamsted potato data the general analysis for 16 treatments tested in quadruplicate in four randomised blocks gave:

Differences due to	Degrees of freedom	Sum of squares	Mean square	Standard error
Blocks	3	5.6307	1.8769	—
Treatments	15	130.2778	8.6852	—
Remainder	45	48.5053	1.0779	1.0382 tons
Total	63	184.4138		

(S.E. of means of four = 0.5191.)

The test of any formula consists in the further analysis of the 15 degrees of freedom due to treatments into two parts representing the portion expressible in the type of formula tested and the residuum not so expressible. If the fit is good and the deviation from the formula may be regarded as due solely to chance, the mean square of this residuum will not be significantly larger than that due to field error, as found above from the 45 degrees of freedom. The General Formula involves 6 degrees of freedom leaving 9 for deviations from the formula. Similarly in the Special Formula after filling the four constants, the deviations from the Special Formula account for the remaining 11 degrees of freedom. The mean squares due to the deviations from the General and the Special Formulae, when compared with the mean squares due to parallels, show that the yields obtained from either of these agree very closely with the observed yields. The sum of squares corresponding to the 9 and 11 degrees of freedom were obtained by multiplying by 4 the values of the expression  $\Sigma (y - m)^2$  calculated from the yields as expected from the General and Special Formulae, since the yields we have been using so far are based upon the means of four plots each.

*Analysis of Variance to show the residuum due to General and Special Formulae.*

Differences due to	Degrees of freedom	Sum of squares	Mean square
Parallels	45	48.5053	1.078
General Formula	6	124.914	20.819
	9	5.364	.596
Special Formula	4	118.076	29.519
	11	12.199	1.109

It is evident that there is no significant deviation from either the General or the Special Formula.

*Standard errors of the constants.* The standard errors of the values of  $a_n$ ,  $a_k$ ,  $n$  and  $k$  were evaluated by the method of maximum likelihood (5) as developed in Appendix I, where it is shown that

$$V(a_k) = \sigma^2 \frac{\begin{vmatrix} \sum_{t=1}^4 \frac{w_t}{(\bar{k} + K_t)^4} & - \sum_{t=1}^4 \frac{w_t}{(\bar{k} + K_t)^2} \\ - \sum_{t=1}^4 \frac{w_t}{(\bar{k} + K_t)^2} & \sum_{t=1}^4 w_t \end{vmatrix}}{\begin{vmatrix} \sum_{t=1}^4 \frac{w_t}{(\bar{k} + K_t)^2} & - \sum_{t=1}^4 \frac{w_t}{(\bar{k} + K_t)^3} & \sum_{t=1}^4 \frac{w_t}{\bar{k} + K_t} \\ - \sum_{t=1}^4 \frac{w_t}{(\bar{k} + K_t)^3} & \sum_{t=1}^4 \frac{w_t}{(\bar{k} + K_t)^4} & - \sum_{t=1}^4 \frac{w_t}{(\bar{k} + K_t)^2} \\ \sum_{t=1}^4 \frac{w_t}{\bar{k} + K_t} & - \sum_{t=1}^4 \frac{w_t}{(\bar{k} + K_t)^2} & \sum_{t=1}^4 w_t \end{vmatrix}}$$

$$\text{and } V(k) = \frac{\sigma^2}{a^2} \left| \begin{vmatrix} \sum_{t=1}^4 \frac{w_t}{(\bar{k} + K_t)^2} & \sum_{t=1}^4 \frac{w_t}{\bar{k} + K_t} \\ \sum_{t=1}^4 \frac{w_t}{\bar{k} + K_t} & \sum_{t=1}^4 w_t \end{vmatrix} \right| \div D$$

(where  $D$  is the denominator of the previous fraction).

The calculation of the values of  $V(a_k)$  and  $V(k)$ , and therefrom of  $\sigma(a_k)$  and  $\sigma(k)$ , was much facilitated by preparing the following table:

$w_t$	$\bar{k} + K_t$	$w_t/\bar{k} + K_t$	$w_t/(\bar{k} + K_t)^2$	$w_t/(\bar{k} + K_t)^3$	$w_t/(\bar{k} + K_t)^4$
24,541	·48	51,127	106,514	221,904	462,300
37,875	1·48	25,591	17,291	11,683	7,894
40,809	2·48	16,455	6,635	2,675	1,079
40,981	4·48	9,148	2,042	456	102
144,206		102,321	132,482	236,718	471,375

The values of  $\sigma(a_k)$  and  $\sigma(k)$  are found to be ·0129 and ·597, which are rather too big and point out the necessity of more accurate data to evaluate the quantities  $a_k$  and  $k$ . Similarly the values of  $\sigma(a_n)$  and  $\sigma(n)$  were found to be ·0616 and ·852 respectively.

(e) *Practical applications of the Resistance Formula.*

The yield curve obtained by the variation of one single factor can with more or less accuracy be represented by any number of algebraic relations, but all these suffer from the inherent weakness that the constants involved will only fit the particular experiment from which these have been evaluated. These constants are thus of no practical use since they cannot be applied to predict the yields in other experiments. Moreover no physical interpretation of these constants can be made. It

is here in its practical applications and the physical interpretation of the constants involved that the strength of the Resistance Formula lies.

The constants  $n$ ,  $k$ , etc. measure the soil fertility in respect of nitrogen, potash, etc. in terms of these manures applied, *i.e.* in cwt. of sulphate of ammonia or potash applied per acre. They do not measure the quantity of plant food present in the soil but what is of more immediate use, *viz.* the quantity of plant food actually available in the soil for the use of the plant. The difference between the two values of  $k$  obtained from the two sets of data from Seale-Hayne (see Appendix II) comes out to be equivalent to nearly 1.2 cwt. of sulphate of potash per acre, which is equivalent to about 70 lb. of  $K_2O$  per acre. Now these two experiments were carried out on similar soil in the same year with the only difference that in the case of one experiment ten tons of dung per acre were added as farmyard manure, while the other field was unmanured. The potash added through ten tons of dung is equivalent to about 112 lb. of  $K_2O$  per acre (7), out of which 70 lb., or nearly 62 per cent., becomes available as plant food the very first year. In the case of nitrogen, however, out of 110 lb. added to every acre through ten tons of dung only 24 lb. became available as plant food in the first year. This difference in the behaviour of potash and nitrogen added to the soil in ten tons of farmyard manure agrees very well with some experiments conducted in Germany (8) on the recovery of potash and nitrogen in potatoes (78 per cent. and 36 per cent.). In the case of the more precise pot cultures (see Appendix II) the calculated values of  $p$  and  $n$  agree still more closely with the facts. From the analysis of the barley plant raised in the data referred to above it was estimated that when only 5 mg. of phosphoric anhydride were added to the pot about 26.1 mg. were recovered in the plant. This surplus of 21.1 mg. must have come from the unmanured sand and is sufficiently near the figure 16.4 mg. obtained by fitting the Resistance Formula to the yields as available phosphorus present in the sand before any manure was added to it, if we took into consideration that the standard error of  $p$  is about 3.2. Although the sand was washed free of nitrogen, during the course of the experiment a certain amount must have been added in the rain and the seed. For the months of March to August at Rothamsted the average nitrogen in the rain is about 2 lb. per acre which is 11 mg. to a 10-inch pot. The seed must supply very nearly 1 mg. each. Nine were sown but only three plants retained, the remaining six being in most cases at least eradicated. We may thus account for 11 mg. from rain and 3 mg. or a trifle more in the seed, against 13.95 mg. indicated by the Resistance Formula. This method of measuring the amount of any

nutrient available to the crop makes direct use only of the behaviour of the plant itself, whereas any laboratory method leaves a great deal of uncertainty as to the availability of the substances measured. It is believed that this method might prove to be of great value for the purposes of soil survey.

The other set of constants  $a_n$ ,  $a_k$ ,  $a_p$ , etc. seem to determine the importance of the manure to the crop or it may be the capacity of the crop to recover the particular manurial nutrient out of the soil. It is believed that these values depend upon the nature of the crop and the variety of the crop as well. Whether the nature or composition of the soil exerts any influence upon these constants cannot yet be investigated for want of suitable data. The values .098, .168 and .079 cwt. of sulphate of ammonia per ton of potatoes obtained for  $a_n$  in all the three potato experiments seem to be quite as close to each other as the standard errors allow us to expect. They are also of the same order as the minimum nitrogen content of potatoes. Kellner gives nitrogen percentages down to 0.23, which is equivalent in our units to 1.20 lb. sulphate of ammonia per ton of potatoes under nitrogen starvation conditions. Similarly the value of  $a_n$  from the barley pot culture (8.204) is pretty close to the mg. of nitrogen recovered per gm. of barley crop shown by the analysis of crop (about 7) under nitrogen starvation conditions. A similar agreement is found in the case of  $a_p$  (1.13) with the amount of phosphorus expressed as  $P_2O$  per gm. dry weight in barley under phosphorus starvation conditions. The practical value of examining more fully the interpretation of these constants cannot be over-emphasised, for these would give us both the optimum value of a single nutrient for any crop as well as the "balanced manure" in case of two or more nutrients for a particular crop and soil. An examination of the manner in which the standard error depends on the design of the experiment would help us in designing more accurate experiments and would thus enable us to determine the constants with greater precision. This knowledge might help us to find the most suitable levels of manures to be used in any experiment and might also throw some light on the possibility of an increase in accuracy being obtained by increasing replications at one level at the cost of another level.

#### 4. SUMMARY AND CONCLUSIONS.

The study of the relation of plant-growth to environmental factors has led to much research directed to the elaboration of General Formulae expressing the quantitative response of the experimental plant or crop

to the quantity of the nutrients with which it is supplied. For variations of a single nutrient only many different mathematical expressions will serve to describe the facts to the accuracy with which these are usually ascertained by experiment; the practical value of such formulae is, however, much impaired if the parameters or constants which they involve change their value from experiment to experiment. If, on the contrary, we can obtain formulae of a general character which represents satisfactorily not only the response to variation of a single factor, but the response to simultaneous variation of two or more different factors, then we have reason to believe that the parameters of such formulae will not depend upon the casual or non-essential conditions of the experiment, but will be capable of direct interpretation as physical quantities. We have shown that the Resistance Formula does fit the data of several two-factor experiments and the agreement of the three values of  $a_n$  determined from the three potato crops as well as the agreement of the difference in the values of  $k$  on a dunged and undunged plot with the potash expected to be available from the ten tons of dung shows that this expectation is so far justified. The parameters of the Resistance Formula are capable of a direct and definite physical interpretation; for each nutrient there are two constants; one represents the importance of the nutrient considered to the crop concerned, and may be expected to vary from crop to crop and from variety to variety, and so to afford a direct comparison between varieties of their manurial needs, while the second represents the amount of nutrient available in the unmanured soil. The practical importance of this second parameter is also very great, for no chemical determination can be relied upon to evaluate the available nutrients, whereas this method renders it possible to evaluate this quantity directly by means of field experiments.

It is shown (a) that it is possible to fit the Resistance Formulae described above to experimental data involving the simultaneous variation of two factors by a sufficiently rapid method of approximation; (b) that in every case discussed the formula fits the facts within the limits of experimental error estimated from the experiments themselves, although formulae of other types fail strikingly to do so. This fact is strikingly borne out from the analysis of variance worked out for the several sets of data; (c) that the parameters appropriate to each nutrient are therefore independent of the abundance of other nutrients and are capable of direct physical interpretation; (d) that even in the best experiments available the sampling errors are too large to allow of estimates of available nutrients to a desirable degree of accuracy, and that greater

precision is also to be desired in the importance factor. This will be possible not only by increased replication, but also with the same number of replicates by an adjustment of the amounts of manure employed as well as by unequal replication of plots with different amounts of manure depending upon the special requirements of the problem; (e) that these constants have great practical value in as much as a knowledge of these would enable us to determine the optimum value of a nutrient for any crop under a given set of conditions. The knowledge of these constants would also help us to recommend an economic and balanced manure containing two or more nutrients for a given crop and soil; (f) it is worth mentioning here that if for certain crops  $a_n$  could be taken as known from the composition of the crop (i)  $n$  could be determined with increased precision, (ii) would be determinate from single dose experiments, and (iii) it would be possible to try an improved type of special formulae, *i.e.*

$$F(N) = \frac{a_n}{n + N} - \frac{b_n}{(n + N)^2}.$$

It is not claimed, nor is it considered probable, that the formulae here investigated represent exactly and in all cases the response of the crop to added nutrients. What is claimed is that the method is capable of supplying information of immediate importance both about soil and about varieties and that only by much increased experimental precision can we hope to exhaust its possibilities or to discover in what respects further improvement is possible.

My best thanks are due to Dr R. A. Fisher of the Rothamsted Experimental Station, who suggested the problem, supplied most of the data and gave valuable guidance at every important stage. My thanks are also due to Dr Gregory of the Imperial College of Science and the authorities of the Seale-Hayne Agricultural College for kindly allowing me to make use of their data.

## 5. APPENDIX I.

Standard errors of the constants:

Maximum likelihood is given by

$$L = -\frac{1}{2\sigma^2} S (y - m)^2,$$

where  $y$  and  $m$  stand for the observed and expected yields respectively.

$$\therefore \frac{\delta L}{\delta a} = \frac{1}{\sigma^2} S \left\{ (y - m) \frac{\delta m}{\delta a} \right\},$$

$$\begin{aligned} \text{or} \quad -\frac{\delta^2 L}{\delta a^2} &= \frac{1}{\sigma^2} S \left\{ -(y - m) \frac{\delta^2 m}{\delta a^2} + \left( \frac{\delta m}{\delta a} \right)^2 \right\} = \frac{1}{V(a)} \\ &= \frac{1}{\sigma^2} S \left\{ \left( \frac{\delta m}{\delta a} \right)^2 - (y - m) \frac{\delta^2 m}{\delta a^2} \right\}, \end{aligned}$$

$$\left[ m = \frac{1}{c + \frac{a_n}{n + N}}, \quad \frac{\delta m}{\delta a} = -m^2 \cdot \frac{1}{n + N} \text{ and } \left( \frac{\delta m}{\delta a} \right)^2 = \frac{m^4}{(n + N)^2} \right].$$

The second term disappears, since the expectation of  $y$  equals  $m$ . Hence

$$-\frac{\delta^2 L}{\delta a^2} = \frac{1}{\sigma^2} S \left[ \left( \frac{\delta m}{\delta a} \right)^2 \right] = \frac{1}{\sigma^2} S \frac{m^4}{(n + N)^2},$$

$$\therefore \frac{1}{V(a)} = \frac{1}{\sigma^2} S \frac{W}{(n + N)^2} \quad \text{.....(i).}$$

$$\text{Similarly} \quad \frac{\delta L}{\delta n} = \frac{1}{\sigma^2} S \left\{ (y - m) \frac{\delta m}{\delta n} \right\},$$

$$-\frac{\delta^2 L}{\delta n^2} = \frac{1}{\sigma^2} S \left\{ -(y - m) \frac{\delta^2 m}{\delta n^2} + \left( \frac{\delta m}{\delta n} \right)^2 \right\} = \frac{1}{V(n)},$$

$$\left[ m = \frac{1}{c + \frac{a_n}{n + N}}, \quad \frac{\delta m}{\delta n} = -m^2 \cdot \frac{-a}{(n + N)^2} \right].$$

$$\therefore \frac{1}{V(n)} = \frac{1}{\sigma^2} S \left[ \left( \frac{\delta m}{\delta n} \right)^2 \right] = \frac{a^2}{\sigma^2} S \frac{W}{(n + N)^4} \quad \text{.....(ii).}$$

$$\text{In the same way} \quad \frac{1}{V(c)} = \frac{1}{\sigma^2} S \left[ \left( \frac{\delta m}{\delta c} \right)^2 \right] = \frac{1}{\sigma^2} S \frac{W}{(n + N)^2} \quad \text{.....(iii),}$$

$$\frac{1}{V(a, n)} = -\frac{\delta^2 L}{\delta a \delta n} = -\frac{a}{\sigma^2} S \frac{W}{(n + N)^3} \quad \text{.....(iv),}$$

$$\frac{1}{V(a, c)} = -\frac{\delta^2 L}{\delta a \delta c} = \frac{1}{\sigma^2} S \frac{W}{n + N} \quad \text{.....(v),}$$

$$\frac{1}{V(n, c)} = -\frac{\delta^2 L}{\delta n \delta c} = -\frac{a}{\sigma^2} S \frac{W}{(n + N)^2} \quad \text{.....(vi).}$$

If  $c$  and  $n$  were known *a priori*, the sampling variance of  $a$  would be  $\frac{1}{L_{aa}}$ , but if  $c$  and  $n$  are determined from the observations (6),

$$V(a) = \begin{vmatrix} L_{nn} & L_{nc} \\ L_{nc} & L_{cc} \end{vmatrix} \div \begin{vmatrix} L_{aa} & L_{an} & L_{ac} \\ L_{an} & L_{nn} & L_{nc} \\ L_{ac} & L_{nc} & L_{cc} \end{vmatrix},$$

where  $L_{aa} = -\frac{\delta^2 L}{\delta a^2}$ ,  $L_{an} = -\frac{\delta^2 L}{\delta a \delta n}$ , etc.

Substituting the values of  $L_{aa}$ ,  $L_{an}$ , etc. we get

$$V(a) = \frac{\begin{vmatrix} a^2 \frac{1}{\sigma^2} \frac{W}{n+N} & -\frac{a}{\sigma^2} \frac{1}{\sigma^2} \frac{W}{(n+N)^2} \\ -\frac{a}{\sigma^2} \frac{1}{\sigma^2} \frac{W}{(n+N)^2} & \frac{1}{\sigma^2} S W \end{vmatrix}}{\begin{vmatrix} \frac{1}{\sigma^2} \frac{1}{\sigma^2} \frac{W}{(n+N)^2} & -\frac{a}{\sigma^2} \frac{1}{\sigma^2} \frac{W}{(n+N)^3} & \frac{1}{\sigma^2} \frac{1}{\sigma^2} \frac{W}{n+N} \\ -\frac{a}{\sigma^2} \frac{1}{\sigma^2} \frac{W}{(n+N)^3} & \frac{a^2}{\sigma^2} \frac{1}{\sigma^2} \frac{W}{n+N} & -\frac{a}{\sigma^2} \frac{1}{\sigma^2} \frac{W}{(n+N)^2} \\ \frac{1}{\sigma^2} \frac{1}{\sigma^2} \frac{W}{n+N} & -\frac{a}{\sigma^2} \frac{1}{\sigma^2} \frac{W}{(n+N)^2} & \frac{1}{\sigma^2} \frac{1}{\sigma^2} W \end{vmatrix}}.$$

Similarly

$$V(n) = \begin{vmatrix} L_{aa} & L_{ac} \\ L_{ac} & L_{cc} \end{vmatrix} \div \begin{vmatrix} L_{aa} & L_{an} & L_{ac} \\ L_{an} & L_{nn} & L_{nc} \\ L_{ac} & L_{nc} & L_{cc} \end{vmatrix} \text{ etc.}$$

## 6. APPENDIX II.

### POTATOES (KERR'S PINK), STACKYARD FIELD, 1926.

*Average yield in tons per acre (y).*

Cwt. per acre sulphate of potash

		0	1	2	4
Cwt. per acre sulphate of ammonia	0	7.80	7.80	8.01	7.79
	1	7.73	8.98	9.17	9.01
	2	9.40	10.56	10.30	10.44
	4	9.53	11.15	11.62	12.34

*Reciprocals of expectations from Special Resistance Formula.*

·14070	·12817	·12575	·12415	·02937
·11977	·10724	·10482	·10322	·00844
·11007	·09754	·09512	·09352	·00126
·10084	·08831	·08589	·08429	·01049
·11133	·09880	·09638	·09478	

*Expectations from Special Resistance Formula (m).*

7.10	7.80	7.95	8.05
8.35	9.33	9.54	9.69
9.08	10.25	10.51	10.69
9.92	11.32	11.64	11.86

$$\Sigma (y - m)^2 = 2.3832$$

$$k = 0.48 \pm 0.60$$

$$n = 1.738 \pm 0.85$$

$$a_k = 0.0089 \pm 0.0129$$

$$a_n = 0.0986 \pm 0.0616$$

Analysis of variance	Degrees of freedom	Sum of squares	Mean square
Field error	45	48.510	1.078
General Formula—Treatments	6	124.914	20.819
	9	5.364	.596
	15	130.278	
Special Formula—Treatments	4	118.076	29.936
	11	12.199	.866
	15	130.275	

POTATOES (SEALE-HAYNE). (Dunged area, 10 tons of dung per acre.)

*Average yield in tons per acre (y).*

Cwt. per acre sulphate of ammonia

Cwt. per acre sulphate of potash		Cwt. per acre sulphate of ammonia			
		0	1	2	3
	0	4.85	6.18	7.38	7.37
	1	5.19	6.72	7.82	8.23
	2	5.46	6.81	7.37	7.95
	3	5.79	7.01	7.85	9.00

*Reciprocals of expectations from Special Resistance Formula.*

.19810	.16008	.14281	.13294	.01179
.18741	.14939	.13212	.12225	.00110
.18355	.14553	.12826	.11839	-.00276
.18155	.14353	.12626	.11639	-.00476
.18631	.14829	.13102	.12115	

*Expectations from Special Resistance Formula (m).*

5.05	6.26	7.01	7.54
5.34	6.70	7.58	8.19
5.45	6.87	7.79	8.45
5.50	6.96	7.91	8.58

$$\Sigma (y - m)^2 = .9910 \text{ in tons per acre}$$

$$= 776.94 \text{ in lb. per plot}$$

$$k = 1.13 \pm 0.58$$

$$n = 1.66 \pm 0.40$$

$$a_k = 0.0258 \pm 0.0170$$

$$a_n = 0.1689 \pm 0.0480$$

Analysis of variance (in lb. per plot)	Degrees of freedom	Sum of squares	Mean square
Field error	45	19,260	428
General Formula—Treatments	6	59,712	9,952
	9	1,610	179
	15	61,322	
Special Formula—Treatments	4	58,216	14,554
	11	3,102	282
	15	61,318	

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POTATOES (SEALE-HAYNE). (Undunged area, 1927.)

*Average yield in tons per acre (y).*

Cwt. per acre sulphate of ammonia

		0                      1                      2                      3			
Cwt. per acre sulphate of potash	0	3.20	4.30	4.13	4.56
	1	3.83	5.74	7.40	6.92
	2	3.68	5.11	6.92	6.91
	3	4.10	5.64	6.52	6.86

*Reciprocals of expectations from Special Resistance Formula.*

·33420	·25125	·23207	·22351	·07578
·24931	·16763	·14818	·13962	·00811
·25470	·17202	·15257	·14401	·00372
·25606	·17338	·15393	·14537	·00236
·25842	·17574	·15629	·14773	

*Expectations from Special Resistance Formula (m).*

2.99	3.98	4.31	4.47
4.01	5.97	6.75	7.16
3.93	5.81	6.55	6.94
3.91	5.77	6.50	6.88

$$\Sigma (y - m)^2 = 1.4965 \text{ in tons per acre} \\ = 750.87 \text{ in lb. per plot}$$

$$k = -.10 \pm .09$$

$$n = .57 \pm .22$$

$$a_k = -.0075 \pm .0064$$

$$a_n = -.0788 \pm .0341$$

Analysis of variance (in lb. per plot)	Degrees of freedom	Sum of squares	Mean square
Field error	45	14,549	323.3
General Formula—Treatments	6	55,298	9216.0
	9	1,726	191.8
	<u>15</u>	<u>57,024</u>	
Special Formula—Treatments	4	53,920	1348.0
	11	3,103	273.0
	<u>15</u>	<u>57,023</u>	

BARLEY POT CULTURES, ROTHAMSTED, 1927.

*Average yields in gm. (dry weight) per pot.*

Nitrogen, mg. per pot

		15	45	105	405	1215
P <sub>2</sub> O <sub>5</sub> in mg. per pot	5	3.06	4.30	11.33	14.84	15.00
	15	3.36	5.77	12.44	19.38	17.88
	45	3.03	6.34	13.34	22.41	29.60
	105	3.39	6.64	14.33	36.30	68.10
	405	3.60	6.30	14.63	33.70	86.40

*Reciprocals of expectations from Resistance Formula.*

·33967	·19545	·11136	·07586	·06296	·04400
·32278	·17856	·09447	·05897	·04607	·02711
·30514	·16092	·07683	·04133	·02843	·00947
·29418	·14996	·06587	·03037	·01747	·00149
·28939	·14517	·06108	·02558	·01268	·00628
·29567	·15145	·06736	·03186	·01896	

*Expectations from Resistance Formula (n).*

2.94	5.12	8.98	13.18	15.88
3.10	5.60	10.59	16.96	21.71
3.28	6.22	13.02	24.20	35.17
3.40	6.67	15.18	32.93	57.24
3.46	6.89	16.37	39.09	78.86
$n = 13.95$		$p = 16.36$		
$a_n = 8.204$		$a_p = 1.131$		

Analysis of variance	Sum of squares	Degrees of freedom	Mean square (mean of 6.24 pots)
$1/\bar{y}^2 S (y - \bar{y})^2$	3.3374	142	.00376
General formula $S (\log y - \log m)^2$	0.0472	16	.00295
Special formula $S (\log y - \log m)^2$	0.0607	19	.00319

## REFERENCES.

- (1) MITSCHERLICH, E. A. *Landw. Jahrb.* **49**, 335, 1916, to **58**, 1923.
- (2) BRIGGS, G. E. Plant Yield and the Intensity of External Factors. *Ann. Bot.* **155**, July 1925, 475-502.
- (3) LEMMERMAN, O. *Zeitschrift für Pflanzenernährung, Düngung und Bodenkunde*. VII Jahrgang, Februar 1928, Heft 2.
- (4) FISHER, R. A. (1921). An Examination of the Yield of Dressed Grain from Broadbalk. *J. Agric. Sci.* **11**, 107-135. (Rothamsted Memoirs, 11.)
- (5) — (1928). *Statistical Methods for Research Workers*. Oliver and Boyd.
- (6) — (1921). On the Mathematical Foundations of Theoretical Statistics. *Phil. Trans. A*, **222**, 309-368.
- (7) RUSSELL, E. J. and RICHARDS, E. H. (1916). On making and storing farmyard manure. *J. Roy. Agric. Soc. Eng.* **77**, 9.
- (8) SCHEIDEWIND, Prof. Dr W. (1922). *Die Ernährung der Landwirtschaftlichen Kulturpflanzen*, pp. 244-245.

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## STUDIES IN CROP VARIATION.

### VI. EXPERIMENTS ON THE RESPONSE OF THE POTATO TO POTASH AND NITROGEN.

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(With Four Text-figures.)

In a previous paper<sup>(1)</sup> the authors have described in detail the design, statistical calculations, and advantages of a method of field experimentation which, on its theoretical side, is based upon the analysis of variance<sup>(2)</sup>. The method is capable of expansion and elaboration in several directions, and the purpose of this paper is to put on record three further examples of experiments in which the new technique has been employed.

As before, it has seemed to us best to present the details of this method in the form of an actual description of experiments themselves rather than as abstract examples. Such a procedure has in this case an added advantage since the three examples chosen follow one another logically, and were each the result of a realisation of both the limitations and the advantages of prior attempts. A consideration of these trials will, it is hoped, enable the experimenter to appreciate the advantages of planning his experiments so as not only to embody an agricultural question, but also to ensure the most accurate decision possible.

When the time was opportune for applying in practice some of the advances in experimental method then available, two of the most important investigations being carried out in the field at Rothamsted, were concerned with

- (1) the qualitative aspect of potash manuring,
- (2) the interaction of potash and nitrogen.

The crop selected was the potato, and it was to these investigations that the new methods were first applied. In the first two years the variety was "Kerr's Pink" and in 1927 "Arran Comrade." The qualitative aspect of the investigation gave rise to a design which may be designated Type I.

#### TYPE I.

The problem at hand was to design an experiment capable of distinguishing the differential effects (if any) of potash applied as sulphate,

muriate, and low grade salt (*i.e.* a sylvenite containing, in addition to potassium chloride, a high percentage of sodium chloride). There were thus three treatments, to which a fourth was added with no potash, this being in the nature of a safeguarding plot to ensure that an apparent equality in the efficacy of the three forms of potash was not in reality due to non-effectiveness and consequent lack of response.

The necessity of a high standard of accuracy to distinguish between equivalent dressings of various forms of the same nutrient together with the smaller number of comparisons attempted, led to the adoption of the Latin square arrangement of plots. The principal features of the design have been described elsewhere(2) but may be repeated here.

Each Latin square experiment contains as many replications as there are treatments. In each row and in each column of the square, each treatment occurs once and once only. It is in this respect that it is a square, for in actual shape it may vary from a true square to a rather pronounced rectangle. The actual allocation of the position of any treatment within its row or column is, apart from this one restriction, determined by chance. It thus follows that for a 4 by 4 Latin square, for instance there are 576 alternative arrangements. An actual experiment is a random choice from the total possible arrangements. Though not completely randomised with respect to plot arrangement, this design possesses complete randomness with respect to the elements of variation used in testing significance. Fig. 1 shows the actual arrangements employed during the two years of the trial. The details of the treatment are as follows:

Basal manuring: Superphosphate 6 cwt. per acre; sulphate of ammonia 2 cwt. per acre.

Potash in the form of sulphate, muriate or low grade salt: The equivalent of 2 cwt. per acre of sulphate of potash.

1925				1926			
M 444	P 422	O 173	S 398	M 584.0	S 557.0	O 461.5	P 498.5
O 279	S 439	M 423	P 409	S 519.5	P 485.5	M 477.0	O 389.0
P 436	M 428	S 445	O 212	P 474.5	O 378.5	S 467.5	M 491.5
S 453	O 237	P 410	M 393	O 464.0	M 511.0	P 507.0	S 492.0

Fig. 1. Arrangement of two Latin squares with yields in lb. per plot.

Applying the analysis of variance to this arrangement it is possible to obtain a variance due to:

- (1) Treatment.
- (2) Position.
- (3) Random variation of parallels.

Table I. *Total yields* (1925).

Rows	Columns	Treatments	
1 1437	1 1612	(O) No potash	901
2 1550	2 1526	(S) Sulphate	1735
3 1521	3 1451	(M) Muriate	1688
4 1493	4 1412	(P) Potash manure salts	1677

Table II. *Analysis of variance* (1925).

Variance due to	Degrees of freedom	Sums of squares	Mean square
Treatments:			
Potash <i>v.</i> no potash	1	119,700	119,700
Potash manures	2	475	237
	— 3	120,175	40,058
Rows	3	1,740	580
Columns	3	5,841	1,947
Parallels	6	1,995	333
Total	15	129,751	—

Table III. *Total yields* (1926).

Rows	Columns	Treatments	
1 2101.0	1 2042.0	(O) No potash	1693.0
2 1871.0	2 1932.0	(S) Sulphate	2036.0
3 1812.0	3 1913.0	(M) Muriate	2063.5
4 1974.0	4 1871.0	(P) Potash manure salts	1965.5

Table IV. *Analysis of variance* (1926).

Variance due to	Degrees of freedom	Sums of squares	Mean square
Treatments:			
Potash <i>v.</i> no potash	1	20,254	20,254
Potash manures	2	1,278	639
	— 3	21,532	7,177
Rows	3	12,055	4,018
Columns	3	3,989	1,330
Parallels	6	2,065	344
Total	15	39,641	—

The positional variance is here of the utmost importance since it can be calculated in two directions, as variance of the rows—*i.e.* variability from top to bottom, and variance of columns (variability from side to side). In this experiment the number of degrees of freedom assigned to each positional factor is three, and since any variation in

column totals does not affect the variation in the row totals, the estimates of the variances of the two are independent, and for the purposes of elimination of positional variances are additive. In other words, a considerable amount more of positional variance can be taken out by using this arrangement as a Latin square than by using it merely as four blocks of four treatments. The actual magnitudes of the variances are given in Tables II and IV.

One point may be mentioned here, which, although not demonstrated by these analyses, is sometimes likely to arise. Treated as a Latin square, the data provide six degrees of freedom for the estimation of error. If the variance of either rows or columns had been sacrificed, there would however have been not six but nine available, and the sums of squares corresponding to the rejected three degrees of freedom would have been absorbed in the remainder sums of squares. The question will sometimes arise as to whether the gain effected by eliminating the sums of squares of either rows or columns will counterbalance the loss entailed by having three less degrees of freedom with which to estimate the random error. In both the examples given it will be seen that even though in 1925 the positional variance of the rows, and in 1926 that of the columns, was small, the error is reduced by eliminating them from the error calculations. But, in cases where this is not so, the fact that the Latin square gives a larger error than blocks with only a one directional variance, has sometimes been held to imply a disadvantage. This is not altogether a fair criticism. One of the merits of the method is the recognition that the arrangement of plots has a real effect upon error estimation, and it makes use of that knowledge. If it were possible to say beforehand that in one particular direction, owing to the absence of a large component of soil heterogeneity in that direction, the positional variance was negligible, then it would be advantageous to use the simpler block arrangement. In point of fact, it is seldom, if ever, with annuals, possible to rely on such a contingency, even where a preliminary uniformity trial has been carried out, and the Latin square arrangement is adopted to make sure that the residual variance, on which hangs the precision of the experiment, shall not be inflated from either source. When, in this way, certain elements of error have been eliminated from the field results, the statistician has no choice but to eliminate them in his estimate of error. To include a portion of these because they make his estimate smaller would be to miss the point of making an unbiased estimate.

These experiments have one defect which in some cases may be

hard to overcome. The inclusion of the no-potash plot (for the reasons specified) does in a year of pronounced response to potash contribute very largely to the treatment variance. In applying tests of significance, particularly what is known as the *z* test, significance may be claimed for treatment as a whole which is really due entirely to the one degree of freedom potash versus no potash. A further analysis of the variance due to treatment by separating this component, as in Tables II and IV, will, of course, settle the point, but the need of this precaution, and the possibility of carrying it out, requires, at the present time, some emphasis.

The benefit of the elimination of the disturbing element of soil heterogeneity is clearly seen in Tables II and IV, if the positional variance is amalgamated with the random variance as it would be if the old methods of designing field experiments were followed. Table V shows this advantage in terms of the standard error per cent., and of the precision figure based thereon.

Table V. *Advantage of eliminating a portion of the soil heterogeneity.*

	Soil variation eliminated	With soil variation	Soil variation eliminated	With soil variation
Standard error %	2.4	3.8	1.9	4.0
Precision	17	7	27	6

In 1925 soil variation would have increased the error by more than 50 per cent. and in 1926 by more than 100 per cent. The influence on the accuracy of an experiment which such an increase of error entails is shown by the figure for precision. This value is arrived at as follows. On a precision scale a 10 per cent. error, the approximate error of a single plot with many crops, is assigned the value 1 and a 1 per cent. error then has a precision value 100, this latter being the value at which with our present resources it is reasonable to aim. The precision index *I* will then be

$$I = 100 \left( \frac{\sigma}{m} \right)^2,$$

where  $\sigma$  is the standard deviation of the mean yield of each treatment, and  $m$  is the mean yield of all.

#### TYPE II.

The Latin square form of experiment answered admirably for the foregoing qualitative distinctions between potash manures, but was unsuited to the investigation into the quantitative relationships between potash and nitrogen.

To serve any useful purpose, this had to include several increments of potash with corresponding increments of nitrogen combined in as many ways as the size of the experiment would allow. To have carried out an experiment involving so large a number of treatments in the form of a Latin square, would have been very wasteful of space and effort. Past a certain point with the Latin square the increase in replication does not bring about a decrease in error commensurate with the labour involved. There are indications that comparisons of more than seven treatments or varieties can be made more precisely with other arrangements. Accordingly, when in 1925 twelve treatments were contemplated, positional variance was eliminated by assigning each treatment to each of four similar blocks, the arrangement of which was substantially the same as that of the top dressing series previously referred to (1). Within the blocks, however, the arrangement of the plots was not a random one.

M 491	N 328	C 340	R 508	D 388	A 322	Block I
L 437	J 217	Q 487	P 464	T 272	S 516	
P 450	S 464	R 461	C 320	N 298	M 482	Block II
T 252	D 352	A 281	L 438	Q 515	J 315	
C 341	P 439	S 456	M 466	J 247	N 344	Block III
T 226	L 393	D 338	R 519	A 198	Q 501	
M 449	A 191	N 185	P 472	D 342	T 234	Block IV
Q 461	R 475	J 157	L 377	C 298	S 441	

Fig. 2. Quantitative experiment of 1925, yields in lb. per plot.

The actual arrangement of the blocks as shown in Fig. 2 was determined by the knowledge that there is a high correlation between adjacent plots.

In 1926 a similar experiment was carried out (Fig. 3). The actual

treatments involved in these trials are shown in the following plan where the letters indicate the treatments employed.

1925					1926				
Sulphate of ammonia, cwt.	Sulphate of potash, cwt.				Sulphate of ammonia, cwt.	Sulphate of potash, cwt.			
	0	2	4	6		0	1	2	4
0	A	C	D	—	0	A	B	C	D
2	J	L	M	—	1	E	F	G	H
4	N	P	Q	R	2	J	K	L	M
6	—	—	—	S	4	N	O	P	Q

N 332.0	J 302.5	F 383.0	A 317.5	D 439.0	O 533.5	K 544.5	A 404.5
K 444.5	Q 568.0	O 450.0	D 381.5	L 483.5	B 308.0	F 434.0	N 468.0
B 363.0	C 368.0	M 449.0	L 471.5	H 422.0	P 500.0	G 402.0	E 318.0
H 447.5	E 314.0	P 527.0	G 434.5	M 504.0	Q 561.5	C 356.0	J 456.0
A 351.5	L 495.5	J 443.0	C 383.5	P 559.0	Q 550.0	B 359.0	E 395.5
K 472.5	B 367.5	G 455.5	O 502.5	C 328.5	H 390.5	J 483.0	O 512.0
E 357.5	F 381.5	Q 531.0	D 316.0	N 522.0	M 444.0	A 325.0	D 259.0
N 385.5	H 354.0	P 496.5	M 474.5	F 410.5	G 351.5	K 430.0	L 394.5

Fig. 3. Quantitative experiment of 1926, yields in lb. per plot.

It was realised that the 1925 experiment was inadequately designed with respect to the treatments included, and the distribution of plots within the blocks, consequently in 1926, by using every possible combination and a strictly random arrangement, the experiment was greatly improved. The analyses of variance are set out in Tables VI and VII and the plan of the arrangements in randomised blocks in Figs. 2 and 3.

Table VI. *Analysis of variance, 1925.*

Variance due to	Degrees of freedom	Sums of squares	Mean square
Treatment	11	464,251	42,205
Blocks	3	22,030	7,343
Parallels	33	34,285	1,039
Total	47	520,566	—

Table VII. *Analysis of variance, 1926.*

Variance due to	Degrees of freedom	Sums of squares	Mean square
Treatment	15	261,497	17,433
Position	3	11,303	3,768
Parallels	45	97,361	2,164
Total	63	370,161	—

The 1926 results call for some explanation. For an experiment in which care has been taken to reduce to a minimum disturbing factors contributing to error, the errors are disconcertingly high. This can be traced to the very small amount of positional variance which has been eliminated. The variance due to position is largely caused by soil heterogeneity, as is also the random variance. The difference between the two lies in the fact that the former is due to systematic changes in fertility affecting whole blocks (inter-block variance), whilst the latter is sporadic in its incidence (intra-block variance). So long as the size of the block is such that the changes of fertility which must occur even in one block are systematic, the variation will be reflected in a large positional variance which is all to the good. If, however, the blocks get so large that within the blocks there is local heterogeneity which is not systematic in incidence, such heterogeneity will increase the remainder or random variance. The question as to how much soil heterogeneity variance makes its appearance in the one or the other sections into which the analysis of variance is divided, depends entirely upon the inter-relation of plot size with block size and the type of soil heterogeneity encountered.

In the present instance it would appear that as only some 10 per cent. of the sum of squares contributable by soil fertility variation is assignable to systematic changes, the blocks have been too large to fulfil their function. Greater replication of smaller blocks would have improved the experiment.

It will be noticed that every experiment of this type really constitutes a sort of uniformity trial in addition to answering the normal agricultural purpose. From a number of experiments carried out on one field over a variety of seasons, a very much fuller knowledge of the behaviour of the field is obtained than could be gained from a similar series of the older type.

#### TYPE III.

The failure to realise the standard of accuracy desired in the 1926 experiment led to further discussion of experimental design and the evolution of a further elaboration. The simple expedient mentioned

above of increasing the replication in order to ensure greater accuracy, was not possible on the potato crop. To have done so would have brought the number of plots in potatoes above the number which could be successfully harvested in the interval between maturity and the onset of bad weather. More plots would have rendered lifting either impossible or at any rate unsatisfactory from the experimental point of view. Any improvement to be effected had to be accomplished without a large increase in plot number, because of this very practical and relevant restriction. The difficulty was overcome by amalgamating the qualitative and quantitative trials. In 1926, these two totalled 80 plots, a Latin square of 16, and 4 blocks of 16. In 1927, the two investigations were combined in an experiment of 81 plots. In order to do this the quantitative side had to be cut down to three increments of nitrogen and potash, but as will appear later there was a marked improvement of accuracy and a much greater fund of information available from the new design.

4 0 333.5	2 0 379.5	2 P4 382.5	2 0 379.0	4 P2 381.0	0 0 382.0	2 0 380.5	0 P4 335.5	2 M2 389.0
0 M4 308.5	2 S2 421.0	4 M2 430.5	4 P4 396.0	4 0 413.5	2 S4 424.5	2 M4 409.5	4 S4 436.0	0 0 348.5
4 S4 403.0	0 0 356.5	0 P2 365.0	0 S2 401.0	2 M2 420.0	0 M4 364.0	4 0 399.0	4 S2 408.0	0 P2 354.0
2 P2 404.5	0 S2 357.0	4 0 412.5	2 P2 408.5	4 S2 438.5	4 0 428.0	4 S4 412.0	2 S2 411.0	0 M2 361.0
4 M2 440.0	2 P4 323.5	0 S4 362.5	2 P4 403.5	2 0 409.5	0 M2 360.5	0 P4 319.0	2 0 402.5	2 M4 369.5
4 M4 436.5	2 0 394.5	0 0 395.0	4 M4 465.5	0 0 366.5	0 S4 395.5	0 0 349.5	4 0 400.5	4 P2 358.5
0 0 337.5	0 M2 345.0	4 0 440.0	2 0 446.5	2 M4 455.0	4 P4 405.5	0 P4 333.0	2 S4 405.0	4 M2 390.5
0 M4 302.0	2 0 377.0	2 S2 467.5	4 S2 473.0	0 P2 395.5	4 0 411.5	0 0 351.5	0 S2 344.0	4 0 369.0
4 P4 356.5	4 P2 388.0	2 S4 463.5	2 M2 474.0	0 0 411.5	0 S4 401.5	2 P2 400.5	2 0 389.5	4 M4 436.0

Fig. 4. Qualitative and quantitative experiment of 1927.

The two numbers in the upper line represent the quantities of nitrogenous and potassic manures, the kind of the latter used being indicated by S for potassium sulphate in cwt. per acre, M for potassium chloride containing equal amount of potassium, and P for the equivalent low grade salt. The lower numbers represent the yield in lb. of a plot of one-fortieth of an acre.

The quantities of potash and nitrogen are shown below:

Sulphate of ammonia, cwt.	Equivalents of sulphate of potash, cwt.		
	0	2	4
0	0 0	0 2	0 4
2	2 0	2 2	2 4
4	4 0	4 2	4 4

and the arrangement in Fig. 4 where S, M, P indicate the source of the potash applied.

These nine treatments constituted the block, and of such blocks there were nine in all. The potash had however to be divided out amongst the three kinds, sulphate, muriate and low grade; there being three plots receiving double and three single potash, one of each in each block were allotted to each kind. The manner of allotting these qualitative differences amongst the varying quantities of nitrogen requires detailed description. The actual position of a plot considered only as representing potash and nitrogen interactions was determined entirely by chance. The element of chance also operated largely in the disposition of the qualitative factor, but there was one restriction. The restriction provided that any particular variety of potash manure should occur in the total of the nine blocks in conjunction with every amount of nitrogen three times. In every other way the distribution was at random.

The amount of replication in this experiment varies with each factor or interaction of factors concerned. The number of independent comparisons which can be made is thus summarised:

	Number of comparisons
(1) Action of potash in varying quantities in combination with a standard quantity of nitrogen	27
(2) Action of nitrogen in varying quantities with standard potash (quantitative)	27
(3) Interaction of nitrogen and potash in every combination	9
(4) Between kinds of potash	18
(5) Differential response of kind of potash to quantity of potash	9
(6) Differential response of kind of potash to quantity of nitrogen	9
(7) Differential response of kind of potash to quantity of potash and nitrogen varying simultaneously	3
(8) Elimination of soil heterogeneity	9

The experiments of 1925 and 1926 gave information on sections 1-4, sections 5-7 are additional information and the accuracy of the comparison between kinds of potash is enormously enhanced, there being now 18 comparisons in place of 4.

The advantage of this type of survey experiment is, as has been pointed out, very great. For each comparison an appropriate error is

obtained with respect to which interpretation can be made. Consideration of the mean yields in conjunction with their appropriate errors shows how greatly improved is the standard of accuracy of the qualitative side of the trial. In 1925, although the means showed the apparent order of efficiency to be sulphate, muriate, low grade salts, even by taking the maximum difference sulphate versus low grade salts (a not entirely fair method), the differences were only probably significant and not completely so, and a similar state of affairs is seen in 1926 with respect to the greatest difference, muriate versus low grade salts.

In the 1927 trial, a summary of which is shown in Table VIII, a much closer control is established and for double dressings the depression of the low grade salts is significant. The depressing effect of muriate rests as a probability and the results show that the effect is felt least where the dressings of nitrogen are high—*i.e.* where the manurial effect of potash would be more apparent. The sulphate appears to function normally at all values of nitrogen.

Table VIII. *Analysis of variance, 1927.*

Variance due to	Degrees of freedom	Sums of squares	Mean square
Potash and nitrogen	8	49,905	6,238
Quality of potash	2	14,458	7,229
Quantity <i>v.</i> quality of potash	2	1,005	503
Blocks	8	21,442	2,680
Error	60	33,919	565
Total	80	120,729	—

## Average yield in tons per acre.

Potash, cwt.	Nitrogen, cwt.			Potash, kind		
	0	2	4	S	M	P
0	6.545	7.061	7.158	6.921	6.921	6.921
2	6.514	7.532	7.357	7.383	7.164	6.858
4	6.193	7.215	7.435	7.348	7.037	6.458

Standard error 0.141 ton.

The analysis of variance shows that differences of decided significance have been obtained both on the quantitative and qualitative questions. The table of average yields shows (i) the responses to increasing dressings of nitrogen, not very large in absolute amount, but capable of fair quantitative estimation in an experiment of the precision actually attained, (ii) a decided response to the first dose of potash, but much less, if any, to the second dose, (iii) that the second dose, when all three kinds of potassic manure are considered together, is deleterious in the absence of nitrogen, but probably becomes beneficial when the total yield is stimulated by heavy nitrogenous dressings.

The table showing the three kinds of potash separately is of special interest in providing unequivocal confirmation of the conclusions indicated, but without sufficient statistical significance, by the earlier experiments; all levels of nitrogenous manuring are here thrown together. With sulphate we have a decided increase from the first dose, and no appreciable decrease due to the second dose. With muriate the yield with double potash is about midway between those obtained with none and with single potash; while with a source of potash which contains much additional sodium chloride, the first dose has on the average no appreciable effect, while the second dose produces a decided loss of yield.

If we contrast the yields at the same level of abundance of potash, we find sulphate beating muriate by 0.22 ton at the single level, and by 0.31 ton at the double level; while it beats the potash manure salts by 0.525 at the single level, and by 0.890 at the double level, the difference being two and one-half to three times as great. It is clear that we must interpret these results, not as due to any difference in availability of the potash, but as due to other effects, presumably the presence of chloride, which effect a quantitative depression of the yield nearly proportional to the quantity of chloride present. The use of no-potash plots designed to show that the crop is really ready to respond to available potash, while essential if availability is in question, are quite superfluous for the examination of effects of this kind, which are most clearly seen with the second dose of potash, to which there is in the present experiment no appreciable response.

#### SUMMARY.

The development is recorded of the series of experiments with potatoes at Rothamsted during 1925-27, designed to examine the quantitative response of yield to varying quantities of nitrogenous and potassic manures, and to test the relative value with this crop of different sources of potash.

While, rather precise comparisons were obtained on the qualitative question by means of Latin squares in 1925-26, the reality of the depression ascribable to chloride could not be demonstrated in these years, but became clearly apparent when in the following year, the qualitative experiment was merged with the quantitative one.

In the earlier quantitative experiments, although satisfactory responses were obtained, the precision of the results left much to be

desired, since only four replicates could be used. When by merging the experiments this was increased to nine replicates, much smaller responses were clearly measurable.

The large and complex type of experiment finally adopted thus supplied more precise information on both heads than could previously be obtained, and in addition to a more thorough exploration of the different combinations possible.

#### REFERENCES.

- (1) T. EDEN and R. A. FISHER. "The experimental determination of the value of top dressings with cereals." *J. Agri. Sci.* (1927) **17**, 548-562.
- (2) FISHER, R. A. *Statistical Methods for Research Workers*. Oliver and Boyd, Edinburgh (1925). 2nd edit. 1928.

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# THE ESTIMATION OF YIELD IN CEREAL CROPS BY SAMPLING METHODS.

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(With Five Text-figures.)

It has long been felt that the use of a reliable sampling method would be highly advantageous for the estimation of yields in experimental work with cereals. Experiments were made during the summer of 1928 with a view to determining the accuracy with which such estimates may be made, and to deciding on a satisfactory sampling technique.

In all cases the number of samples taken from plots whose size varied from about one-seventeenth to one-fifth of an acre, was 30, each sample consisting of the total produce from a metre-length of a single drill. Each sample was tied with string and labelled on the field. Later it was weighed as a whole, after which the heads were cut off and threshed by hand. The grain was then weighed, and the weight corrected for moisture content, so that all calculations were based on dry weight.

Two, and in one case three, sampling methods were tried.

(a) The plots, all of which were narrowly rectangular in shape, were divided transversely into three equal parts, and ten samples were taken at random from each part.

(b) Six sets, each of which comprised a succession of five contiguous metre-lengths, disposed symmetrically within the plot, were cut as samples, each metre-length being tied separately. A somewhat similar scheme—the “Rod-Row Method,”—has been adopted by American agronomists<sup>(1)</sup>, and it was thought desirable to compare it with a method based on random sampling.

(c) Six metre-lengths at equal intervals along the plot were cut from each of five drill-rows chosen at random. This scheme was only tried on one occasion, with wheat. Tables of the primary data will be found in Appendix I.

## I. RESULTS WITH BARLEY.

These plots, of areas one-seventeenth to one-eleventh of an acre, and of three different varieties, were each sampled by methods (a) and (b) above. Total produce and grain were weighed for each metre-length, and results were calculated for straw as well as grain.

The statistical technique known as the "Analysis of Variance" was devised by R. A. Fisher, and first published in its complete form in 1923(5). The principle of the method is that the total variation between the individual results in a set of data, if measured in terms of the sum of squares of deviations of these results from their general mean, may be analysed into a number of parts by the application of a well-known algebraic identity. This allows of the apportioning of fractions of the total sum of squares to various known causal factors, leaving a residual fraction due to unknown or uncontrolled factors. This latter fraction provides a logical basis for an estimate of the errors of an experiment. Fisher has further shown that the mean value of the fraction ascribable to any factor—the "variance"—is obtained by dividing that fraction by the number of "degrees of freedom" on which it is based, where "degrees of freedom" is used in the sense of "independent comparisons." Thus between  $n$  quantities whose mean is fixed there are in general  $n - 1$  independent comparisons or degrees of freedom.

In the following pages the experimental results are treated in turn by this method. There are in all cases 29 degrees of freedom, since 30 samples were taken from every plot. In Method (a), since each plot was divided into three parts, 10 samples being taken from each part, the total variance may be analysed into a portion representing differences between the mean yield of the parts, and a residue representing differences between metre-lengths within the same part. The former portion may fairly be eliminated as being due to differences in mean fertility between the parts; the latter is used for the estimation of experimental error, representing as it does variance due to smaller differences in fertility within each part, to errors of measurement of the metre-lengths, to loss of grain in threshing, to errors in weighing, etc., etc. In a precisely similar manner the variance of results of Method (b) may be divided into two fractions, one due to differences between 5-metre-lengths or "sets," the other due to differences between metre-lengths within the same set. The former would be the basis of the estimate of error were the sets cut and weighed as wholes.

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### ANALYSES OF VARIANCE OF WEIGHTS PER METRE-LENGTH, IN GRAMS.

#### 1. Variety "824."

##### *Method (a). Random sampling.*

##### 1. Grain.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error
Blocks	2	2072.50	—	—
Remainder	27	6491.46	240.42	15.51
Total	29	8563.96		

Diminution\* of variance = 18.59 per cent.

Standard error of a single metre-length = 15.51 gm.

Hence standard error of mean of 30 =  $\frac{15.51}{\sqrt{30}} = 2.83$  gm.

Mean = 47.29 gm.: hence standard error of mean = 5.99 per cent.

##### 2. Straw.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error
Blocks	2	5068.27	—	—
Remainder	27	12331.93	456.74	21.37
Total	29	17400.20		

Diminution of variance = 23.88 per cent.

Standard error of a single metre-length = 21.37 gm.

Hence standard error of mean of 30 =  $\frac{21.37}{\sqrt{30}} = 3.90$  gm.

Mean = 72.97 gm.: hence standard error of mean = 5.35 per cent.

##### *Method (b). Systematic sampling: symmetrical method.*

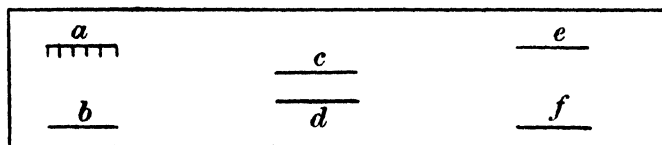


Fig. 1. Plan showing position of "sets" of metre-lengths in Method (b).

##### 1. Grain.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error	"z"
Inter-set	5	1247.10	249.42	(15.79)	
Intra-set	24	2350.75	97.95	—	0.476
Total	29	3597.85	124.06	(11.14)	

Standard error of a single metre-length:

(a) as calculated from whole sets = 15.79 gm.;

(b) as calculated from individual values = 11.14 gm.

\* See p. 230.

Hence standard error of mean:

$$(a) = \frac{15.79}{\sqrt{30}} = 2.88 \text{ gm.} = 7.20 \text{ per cent. of mean;}$$

$$(b) = \frac{11.14}{\sqrt{30}} = 2.04 \text{ gm.} = 5.10 \text{ per cent. of mean.}$$

It is interesting to note that, had the sets been cut as a whole, the standard errors would have been considerably overestimated, owing to the greater variability between than within sets. (Since the arrangement of the metre-lengths was systematic and not random, the standard errors obtained do not provide, in either case, valid estimates of the error of the mean.)

## 2. Straw.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error	"z"
Inter-set	5	6666.08	1333.22	(36.51)	0.9730
Intra-set	24	4570.83	190.45	—	
Total	29	11236.91	387.48	(19.68)	

Standard error of a single metre-length:

(a) as calculated from whole sets = 36.51 gm.;

(b) as calculated from individual values = 19.68 gm.

Hence standard error of mean:

$$(a) = \frac{36.51}{\sqrt{30}} = 6.67 \text{ gm.} = 10.27 \text{ per cent. of mean;}$$

$$(b) = \frac{19.68}{\sqrt{30}} = 3.59 \text{ gm.} = 5.54 \text{ per cent. of mean.}$$

As before there is a much higher variation between than within sets. The significance of this difference is easily found by R. A. Fisher's "z" test. "z" is half the difference between the natural logarithms of the two variances, and its standard error depends only on the number of degrees of freedom on which the variances are based. Tables have been provided<sup>(4)</sup> showing the value of "z" which must be attained for two different levels of significance, the 5 per cent. and the 1 per cent. points. If the 5 per cent. point of "z" is reached, it is to be understood that as great a difference between the two variances as was actually observed, would only occur by chance, from homogeneous material, once in 20 samples. Taking the 5 per cent. point, then, as a convenient minimum level for significance, the difference here found is hardly significant in the case of the grain, but highly significant with straw. The 5 per cent. point of "z" is 0.4817, and the 1 per cent. point, 0.6799.

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### 2. Variety "Spratt Archer."

*Method (a). Random sampling.*

#### 1. Grain.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error
Blocks	2	1195.27	—	—
Remainder	27	6778.17	251.04	15.84
Total	29	7973.44		

Diminution of variance = 9.70 per cent.

Standard error of a single metre-length = 15.84 gm.

Hence standard error of mean =  $\frac{15.84}{\sqrt{30}} = 2.89$  gm. = 5.72 per cent.

of mean.

#### 2. Straw.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error
Blocks	2	2871.63	—	—
Remainder	27	19112.13	707.86	26.61
Total	29	21983.76		

Diminution of variance = 6.62 per cent.

Standard error of a single metre-length = 26.61 gm.

Hence standard error of mean =  $\frac{26.61}{\sqrt{30}} = 4.86$  gm. = 6.09 per cent.

of mean.

*Method (b). Systematic sampling: symmetrical method.*

#### 1. Grain.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error	"z"
Inter-set	5	1568.31	313.66	(17.71)	
Intra-set	24	4203.37	175.14	—	0.2914
Total	29	5771.68	199.02	(14.11)	

Standard error of a single metre-length:

(a) as calculated from whole sets = 17.71 gm.;

(b) as calculated from individual values = 14.11 gm.

Hence standard error of mean:

$$(a) = \frac{17.71}{\sqrt{30}} = 3.23 \text{ gm.} = 6.57 \text{ per cent. of mean;}$$

$$(b) = \frac{14.11}{\sqrt{30}} = 2.58 \text{ gm.} = 5.24 \text{ per cent. of mean.}$$

## 2. Straw.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error	"z"
Inter-set	5	2852.58	570.51	(23.89)	
Intra-set	24	7864.37	327.68	—	0.2773
Total	29	10716.95	369.55	(19.22)	

Standard error of a single metre-length:

(a) as calculated from whole sets = 23.89 gm.;

(b) as calculated from individual values = 19.22 gm.

Hence standard error of mean:

$$(a) = \frac{23.89}{\sqrt{30}} = 4.36 \text{ gm.} = 6.95 \text{ per cent. of mean;}$$

$$(b) = \frac{19.22}{\sqrt{30}} = 3.51 \text{ gm.} = 5.59 \text{ per cent. of mean.}$$

Here, although the inter-set is greater than the intra-set variance, the difference is not great, and falls short of significance when tested by the "z" method.

## 3. Variety "Plumage Archer."

Method (a). Random sampling.

## 1. Grain.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error
Blocks	2	2870.52	—	—
Remainder	27	9319.32	345.16	18.58
Total	29	12189.84		

Diminution of variance = 17.89 per cent.

Standard error of a single metre-length = 18.58 gm.

Hence standard error of mean =  $\frac{18.58}{\sqrt{30}} = 3.39 \text{ gm.} = 7.47 \text{ per cent.}$

of mean.

## 2. Straw.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error
Blocks	2	13502.00	—	—
Remainder	27	24293.65	899.76	30.00
Total	29	37795.65		

Diminution of variance = 30.96 per cent.

Standard error of a single metre-length = 30.00 gm.

Hence standard error of mean =  $\frac{30.00}{\sqrt{30}} = 5.477 \text{ gm.} = 8.31 \text{ per cent.}$

of mean.

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*Method (b). Systematic sampling: symmetrical method.*

### 1. Grain.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error	"z"
Inter-set	5	5733.95	1146.79	(33.86)	0.9842
Intra-set	24	3845.30	160.22	—	
Total	29	9579.25	330.32	(18.17)	

Standard error of a single metre-length:

(a) as calculated from whole sets = 33.86 gm.;

(b) as calculated from individual values = 18.17 gm.

Hence standard error of mean:

$$(a) = \frac{33.86}{\sqrt{30}} = 6.18 \text{ gm.} = 15.77 \text{ per cent. of mean;}$$

$$(b) = \frac{18.17}{\sqrt{30}} = 3.32 \text{ gm.} = 8.46 \text{ per cent. of mean.}$$

### 2. Straw.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error	"z"
Inter-set	5	7924.23	1584.85	(39.81)	0.8012
Intra-set	24	7661.56	319.23	—	
Total	29	15585.79	537.44	(23.18)	

Standard error of a single metre-length:

(a) as calculated from whole sets = 39.81 gm.;

(b) as calculated from individual values = 23.18 gm.

Hence standard error of mean:

$$(a) = \frac{39.81}{\sqrt{30}} = 7.27 \text{ gm.} = 13.78 \text{ per cent. of mean;}$$

$$(b) = \frac{23.18}{\sqrt{30}} = 4.23 \text{ gm.} = 8.03 \text{ per cent. of mean.}$$

The 5 per cent. of "z" is 0.4817, and the 1 per cent. point, 0.6799. The significance of the difference between the intra- and inter-set variance therefore exceeds 1 in 100 both for grain and for straw. The effect of this is seen in the very much higher estimate of standard error obtained from whole sets as compared with individual metre-lengths.

## II. RESULTS WITH WHEAT.

*Variety "Red Standard."**Method (a). Random sampling.*

## 1. Grain.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error
Blocks	2	1562.55	—	—
Remainder	27	8476.48	313.94	17.72
Total	29	10039.03		

Diminution of variance = 9.31 per cent.

Standard error of a single metre-length = 17.72 gm.

Hence standard error of mean =  $\frac{17.72}{\sqrt{30}} = 3.24$  gm. = 8.08 per cent.

of mean.

## 2. Straw.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error
Blocks	2	1122.02	—	—
Remainder	27	74295.45	2751.68	52.46
Total	29	85524.47		

Diminution of variance = 6.69 per cent.

Standard error of a single metre-length = 52.46 gm.

Hence standard error of mean =  $\frac{52.46}{\sqrt{30}} = 9.58$  gm. = 8.72 per cent.

of mean.

*Method (b). Systematic sampling: symmetrical method.*

## 1. Grain.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error	"z"
Inter-set	5	1469.41	293.88	(17.14)	0.4044
Intra-set	24	3141.50	130.90	—	
Total	29	4610.91	159.00	(12.61)	

Standard error of a single metre-length:

(a) as calculated from whole sets = 17.14 gm.;

(b) as calculated from individual values as previous = 12.61 gm.

Hence standard error of mean:

$$(a) = \frac{17.14}{\sqrt{30}} = 3.13 \text{ gm.} = 8.09 \text{ per cent. of mean;}$$

$$(b) = \frac{12.61}{\sqrt{30}} = 2.31 \text{ gm.} = 5.96 \text{ per cent. of mean.}$$

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### 2. Straw.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error	"z"
Inter-set	5	10036.45	2007.29	(44.80)	0.4732
Intra-set	24	18699.00	779.13	—	
Total	29	28735.45	990.88	(31.48)	

Standard error of a single metre-length:

(a) as calculated from whole sets = 44.80 gm.;

(b) as calculated from individual metres = 31.48 gm.

Hence standard error of mean:

$$(a) = \frac{44.80}{\sqrt{30}} = 8.18 \text{ gm.} = 9.04 \text{ per cent. of mean;}$$

$$(b) = \frac{31.48}{\sqrt{30}} = 5.75 \text{ gm.} = 6.35 \text{ per cent. of mean.}$$

In this case the differences between inter- and intra-set variances just fail to reach the 1 in 20 level of significance ("z" = 0.4817) for straw, and is smaller for grain. There is therefore little difference between standard errors based on the two variances.

*Method (c). Systematic sampling: Random Row method.*

### 1. Grain.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error	"z"
Inter-row	4	21922.83	5480.71	(74.03)	1.4109
Intra-row	25	7154.37	286.17	—	
Total	29	29077.20	1002.66	(31.66)	

Standard error of a single metre-length:

(a) as calculated from whole rows = 74.03 gm.;

(b) as calculated from individual metres = 31.66 gm.

Hence standard error of mean, as calculated from individual metre-

$$\text{lengths} = \frac{31.66}{\sqrt{30}} = 5.78 \text{ gm.} = 11.33 \text{ per cent. of mean.}$$

### 2. Straw.

Fraction	Degrees of freedom	Sum of squares	Mean square	Standard error	"z"
Inter-row	4	92529.83	23132.46	—	1.4250
Intra-row	25	33452.49	1338.10	—	
Total	29	125982.32	4344.22	(65.91)	

Standard error of a single metre-length = 65.91 gm.

$$\text{Hence standard error of mean} = \frac{65.91}{\sqrt{30}} = 12.03 \text{ gm.} = 9.67 \text{ per cent.}$$

of mean.

As the very high values of "z" indicate, the variation between rows has been very much greater than within rows. This was largely due to the fact that an edge-row was sampled, and exaggerates somewhat the danger of systematic sampling of this type. Partial choking of drill-coulters, nearness of rows to field-drains, and many other factors, do, however, tend to make rows as a whole differ widely from their neighbours, and add weight to the case for random sampling.

#### RÉSUMÉ OF RESULTS.

The most important result that emerges is that with plots having an area of about one-sixteenth of an acre, a "random sampling" method will provide an estimate of yield with a standard error of less than 6 per cent. when 30 samples of metre-length of drill are taken. This would indicate that with plots one-fortieth of an acre in area, the average standard error should be not more than 5 per cent. It is customary at Rothamsted to have experimental plots of about this area, and since the standard error of such plots arising from causes other than sampling errors has been shown to be about 8-10 per cent., the additional inaccuracy introduced by the use of the sampling method described, will be quite small. Thus a standard error of 8 per cent. is increased to 9.4 per cent., and one of 10 per cent. only to 11.2 per cent. by the superposition of a further error of 5 per cent.

As the figures for "Percentage of variance eliminated" show very clearly, it is of great advantage to divide the area to be sampled into a small number of parts within each of which an equal number of samples is taken. By this means, and by the use of R. A. Fisher's statistical technique, the Analysis of Variance, a substantial reduction in the standard error may be effected.

Certain disadvantages of the systematic methods tried also stand out clearly. In the first place, the "rod-row" method used extensively in America is shown to suffer from the grave defect that the unit is often too coarse. This will be referred to later. Secondly, any attempt to reduce the labour of sampling by taking samples only from a small number of rows, whether these be chosen systematically or at random, is liable to lead to an increased estimate of error owing to the difference between rows as wholes—i.e. to the greater variation between than within rows. On the one occasion on which the method was adopted, the intra-row correlation, easily calculated from the ratio of intra-row and total variance (see R. A. Fisher (4) p. 191), is + 0.6635, and highly

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significant. This indicates that the location of samples was very far from random, and the validity of the estimate of error correspondingly prejudiced.

*Table of results.*

	Crop Variety	Area	Barley "824"		Barley "Spratt Archer"		Barley "Plumage Archer"		Wheat "Red Standard"	
			0.06 acre		0.06 acre		0.09 acre		0.2 acre	
			Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
<i>Method (a)</i>										
Random sampling	Mean weight per metre in grams		47.29	72.97	50.60	79.75	45.41	65.94	40.04	109.92
	Standard error of mean (%)		5.99	5.35	5.72	6.09	7.47	8.31	8.08	8.72
<i>Method (b)</i>										
Systematic sampling: symmetrical method	Mean weight per metre in grams		40.01	64.91	49.25	62.78	39.20	52.74	38.70	90.44
	Standard error of mean calculated from whole sets (%)		7.20	10.27	6.57	6.95	15.77	13.78	8.09	9.04
	Standard error of mean calculated from individual metres (%)		5.10	5.54	5.24	5.59	8.46	8.03	5.96	6.36
<i>Method (c)</i>										
Systematic sampling: "Random row" method	Mean weight per metre in grams		—	—	—	—	—	—	51.00	124.40
	Standard error of mean (%)		—	—	—	—	—	—	11.33	9.67

### DISCUSSION.

Sampling methods have hitherto been employed on numerous occasions for estimating the yields of cereal plots, but in extremely few cases is it possible to gain any idea of the accuracy of the methods used. Perhaps the fullest available sets of data are those of Arny and Garber (1), and of Arny and Steinmetz (2) and it will be instructive to examine these in some detail.

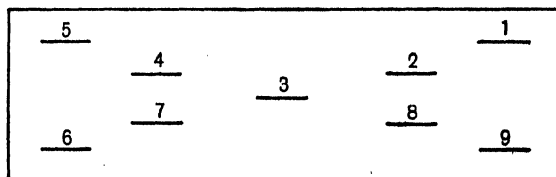


Fig. 2.

Arny and Garber employed the "rod-row" method, cutting nine symmetrically disposed rod-lengths of drill from each plot, and comparing the resulting estimates of yield with those obtained by harvesting the whole plots. The area of the plots was in all cases a tenth of an acre.

The position of the samples was as shown in Fig. 1.

The yield was estimated not only from the mean of all the nine samples but also from four only (samples 2, 4, 7 and 8), and from five only (samples 1, 3, 5, 6 and 9).

In order to obtain some idea of the additional errors introduced by sampling, the figures for "Marquis" wheat grown at the Morris Substation, and for "Haynes Bluestem" wheat grown at University Farm, St Paul, Minn. (Tables III and V in the original paper), have been grouped together, and an analysis made of the total variance. Of the sampling figures only those for nine and for four samples have been considered.

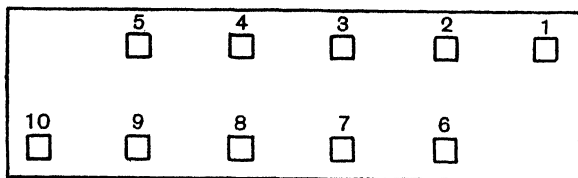


Fig. 3.

Each of the two experiments involved 18 plots, there being six different treatments in triplicate. It is therefore possible to eliminate that portion of the variance due to treatments, thus obtaining a residual variance representing differences between plots similarly treated.

### 1. Analysis of figures for total yields.

Fraction	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}$ log mean square	Standard error	Standard error %
Sets	11	160.23	14.57	—	—	—
Remainder	24	116.73	4.86	0.7907	2.205	6.68
Total	35	276.96				

### 2. Analysis of figures for nine rod-row samples.

Fraction	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}$ log mean square	Standard error	Standard error %
Sets	11	399.17	36.88	—	—	—
Remainder	24	163.11	6.796	0.9583	2.607	7.63
Total	35	562.28				

### 3. Analysis of figures for four rod-row samples.

Fraction	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}$ log mean square	Standard error	Standard error %
Sets	11	299.42	27.220	1.5513	2.861	8.54
Remainder	24	196.50	8.187	—	—	—
Total	35	495.92				

The data of Arny and Steinmetz have been similarly treated. Here the sampling units were square yards instead of rod-rows. Their arrangement is shown in Fig. 3.

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As before yields were calculated not only from the 10 samples from each plot, but also from 9, 8, 5, 4 from the centre, and 4 from the ends. Analyses were made of figures for total yields, for estimates from 10 samples, and from 5 samples. Series II, III and IV, were grouped for this purpose.

### 4. Analysis of figures for total yields.

Fraction	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}$ log mean square	Standard error	Standard error %
Sets	17	2293.73	134.925	—	—	—
Remainder	36	119.38	3.316	0.5994	1.821	6.62
Total	53	2413.11				

### 5. Analysis of figures for ten square-yard samples.

Fraction	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}$ log mean square	Standard error	Standard error %
Sets	17	1116.93	65.702	—	—	—
Remainder	36	186.75	5.188	0.8232	2.278	9.80
Total	53	1303.68				

### 6. Analysis of figures for five square-yard samples.

Fraction	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}$ log mean square	Standard error	Standard error %
Sets	17	1046.06	—	—	—	—
Remainder	36	275.30	7.647	1.0172	2.765	12.01
Total	53	1321.36				

The additional standard error per plot due to sampling may now be estimated for the values of the mean squares given above:

9 rod-rows ...	...	...	...	3.67 per cent.
4 „ ...	...	...	...	5.32 „
10 square yards	...	...	...	7.23 „
5 „	...	...	...	10.03 „

The first three of these standard errors are fairly small, and, since a rod is roughly equivalent to 5 metres, of about the same order as those obtained in the random sampling method. It must be noted, however, that these estimates are subject to very large sampling errors, owing to the fact that they are calculated from the differences between variances.

The standard error of the difference between two variances based on  $N$  degrees of freedom is given by the formula:

$$s_d = \sqrt{\frac{2}{N}(\sigma_1^4 + \sigma_2^4 - 2r_{\sigma_1^2 \sigma_2^2} \sigma_1^2 \sigma_2^2)},$$

where  $\sigma_1^2$ ,  $\sigma_2^2$  are the two variances, and  $r_{\sigma_1^2 \sigma_2^2}$  is the correlation between them in samples.

Now it has recently been shown by Wishart (<sup>(6)</sup> p. 43), that if  $\rho_{1.2}$  is the correlation between the two variates in the original population  $r_{\sigma_1^2 \sigma_2^2} = \rho_{1.2}^2$ , exactly.

Then, substituting for the population parameters  $\sigma_1^2, \sigma_2^2, \rho_{1.2}$ , the observed values  $s_1^2, s_2^2, r_{1.2}$ , which are the best available estimates, we have:

$$s_d = \sqrt{\frac{2}{N} (s_1^4 + s_2^4 - 2r_{1.2}^2 s_1^2 s_2^2)}$$

is in each case the residual correlation between the estimates of yield obtained by the two methods—*i.e.* the correlation calculated from the “Remainder” variances and covariances<sup>1</sup>. The covariances must therefore be analysed in precisely the same manner as the variances, before the correlation coefficients can be obtained.

(a) *Total yields and 9 rod-rows.*

Fraction	Covariance	Correlation coefficient
Sets	150.733	+0.5960
Remainder	89.056	<u>+0.6464</u>
Total	239.789	+0.6076

(b) *Total yields and 4 rod-rows.*

Fraction	Covariance	Correlation coefficient
Sets	154.669	+0.7061
Remainder	105.855	<u>+0.6989</u>
Total	260.524	+0.7030

(c) *Total yields and 10 square yards.*

Fraction	Covariance	Correlation coefficient
Sets	1528.066	+0.9547
Remainder	88.286	<u>+0.5913</u>
Total	1616.352	+0.9113

(d) *Total yields and 5 square yards.*

Fraction	Covariance	Correlation coefficient
Sets	1451.438	+0.9370
Remainder	106.399	<u>+0.5869</u>
Total	1557.837	+0.8724

Using the underlined correlation coefficients, the following results are obtained for the accuracy of the difference between the residual variances:

- (a)  $1.932 \pm 1.878$ ,
- (b)  $3.324 \pm 2.077$ ,
- (c)  $1.872 \pm 1.199$ ,
- (d)  $4.331 \pm 1.699$ .

Hence only the last can be said to differ significantly from zero, and in no case is the value of the sampling error established with any approach to certainty.

The same result may be arrived at more simply by the use of R. A. Fisher's “*z*” transformation, by means of which the significance of a difference between variances may be tested directly.

<sup>1</sup> The “covariance” is the average value of the sum of products of deviations from the mean, and is obtained by dividing that quantity by the appropriate number of degrees of freedom.

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### 1. Arny and Garber's data.

	Total yield and 9 rod-rows	Total yield and 4 rod-rows	5 % point of "z"	1 % point of "z"
"z"	0.1676	0.2606	0.3425	0.4890

### 2. Arny and Steinmetz' data.

	Total yield and 10 square yards	Total yield and 5 square yards	5 % point of "z"	1 % point of "z"
"z"	0.2238	0.4178	0.2596	0.3702

Here again only the last result attains even the 5 per cent. point of "z"—*i.e.* only in the last case would such a difference between variances occur as infrequently as once in 20 samples from a homogeneous population. This confirms the conclusion that little reliance can be placed on the calculated values of the errors due to sampling, and illustrates in a striking manner one of the great disadvantages of a systematic as compared with a random sample. A random sample gives a direct estimate of the errors due to sampling, an estimate, therefore, of far greater accuracy than the indirect estimate obtained as above. It is of great importance that such an estimate should be arrived at, since the improvement of experimental technique depends on a knowledge of the causes of inaccuracy. Data such as those of Arny and Garber do not distinguish adequately between errors due to insufficient replication, and errors of sampling within the plots. If the main sources of error were the former, the taking of a greater number of samples from each plot would do little towards increasing the accuracy of the experiment, and vice versa.

The only manner in which direct estimates of the sampling error of a systematic method can be obtained, is by making a series of observations in which at least duplicate sets of samples are taken from each plot. These sets must further be such that they form a random sample from the whole population of possible sets. Only under these circumstances can a valid estimate of error be made. Hence there is the initial condition that such a population exists, for if it does not exist, no random sample can be made from it, nor can a standard error be calculated. In the present instance it is very difficult to see how such populations can be constructed. It is, however, possible, to devise systematic methods which do admit of the calculation of a valid standard error. The method used by Engledow (3), is a case in point. Here, in one variant of the method, 1-ft. samples are cut as in Fig. 4.

*AB* represents the width of the area to be sampled and is measured parallel with the drill-rows. 1, 2, 3 ... represent the 10th, 20th, 30th ...

drill rows. Samples are taken successively from these rows, there being a constant lateral shift from sample to sample. When a complete traverse of the area has been effected, a fresh start is made from the far side, as on the 70th drill-row in the figure.

If  $n$  samples are taken in passing from side to side of the area, there may be considered to be  $n$  possible starting-points along the base line  $AB$ . It would then be possible to get a valid estimate of error by taking

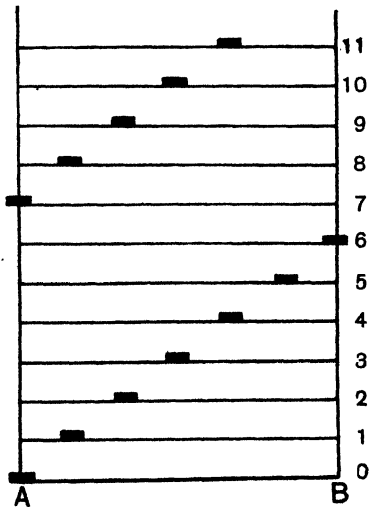


Fig. 4.

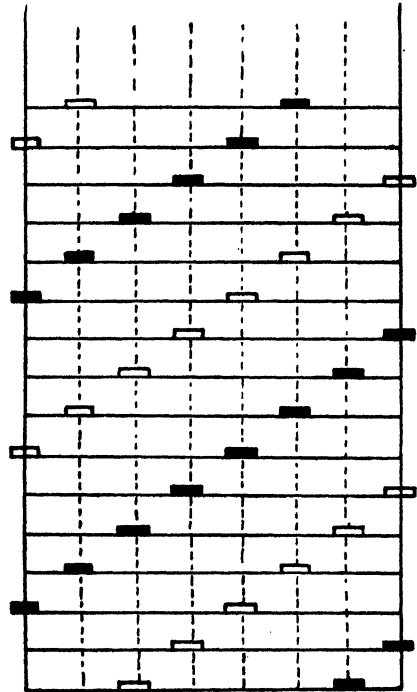


Fig. 5.

duplicate sets from each area to be sampled, the starting-points of the sets being chosen at random from among the  $n$  possible points. Thus in one of the areas, the samples might be taken as in Fig. 5.

A systematic arrangement, such as that of Engledow, samples the area very effectively, but it can scarcely be maintained that this advantage outweighs the disadvantage that the samples do not in themselves yield an estimate of their standard error, as would be the case with a random sample. Thus it should be noted that  $n$  separate sets of systematic samples would be required to yield the same information as to sampling errors as a single set of  $n$  samples distributed at random.

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This is true whatever the number of samples in the systematically arranged set. It can be ensured moreover that the random method does sample the whole area, by dividing the area into a number of subdivisions. Then if the same number of samples is taken from each part of the area, there is secured both an effective distribution of samples, and an arrangement which permits of the elimination of that portion of the total variance which is due to differences in the mean fertility of the subdivisions of the area. This is, of course, equally possible with a systematic method, but although the necessary statistical technique—the Analysis of Variance—has been available for some years, no such use has previously been made of it.

The advantage to be gained by the subdivision of the area to be sampled has already been referred to. It may be useful, however, to collect the figures which bear on the point. Below are given the percentages of the total variance which are eliminated as being due to difference in soil fertility over subdivisions of the areas sampled, and which, consequently, represent percentage reductions of the variance on which the standard errors are based.

Crop	Variety	% reduction in variance	
		Grain	Straw
Barley	"824"	18.59	23.88
"	"Spratt Archer"	9.70	6.62
"	"Plumage Archer"	17.89	30.96
Wheat	"Red Standard"	9.31	6.69

It will be seen that in no case is less than 6.5 per cent. of the variance removed by this procedure, the mean reduction being 15.46 per cent.

With regard to the size and nature of the sampling unit, our results show conclusively that the rod-row is too coarse a unit. The significant intra-class correlations obtained when separate weighings are made of the five metre-lengths in each sampling unit of method (b), are as shown below:

Crop	Variety	Intra-class correlations (where significant)	
		Grain	Straw
Barley	"824"	—	+0.7966
"	"Spratt Archer"	—	—
"	"Plumage Archer"	+0.6886	+0.3856
Wheat	"Red Standard"	—	+0.1866*

\*  $p$  = about 0.053.

Except where otherwise stated the level of significance has been taken as  $p = 0.050$ , where  $p$  is the probability that so high a value could be obtained by chance.

These figures indicate that there has been a considerable loss of information over that provided by the same number of metre-lengths arranged at random over the area. In certain other investigations by the author it has even appeared that the metre may be too long, significant correlations having been obtained between successive half-metre-lengths of drill. In view of this experiments were tried with a dissected 4-ft.-length, each foot being separated from its neighbour by 2 ft. of unsampled corn. No significant intra-class correlations were obtained with this method, even when "neighbouring" foot-lengths were compared. Engledow uses the foot-length as his unit, and points out that smaller lengths would be impracticable owing to the increased importance of end-errors. There being no intra-class correlation between the parts of a dissected 4-ft.-length, it is better to use 30 of such units rather than 120 separately located 1-ft.-lengths, since the location of each of the former units fixes 4 ft. at once, thus reducing the labour involved in sampling.

What has been said of using the rod-row as a unit will be equally true of the square yard. In fact it seems highly probable that the loss of information would be even greater in the case of five metres lying side by side than if they were end to end.

In conclusion, I wish to thank Dr R. A. Fisher of this Station for valuable criticism and advice; and Messrs H. J. Johnson and T. W. Simpson of Armstrong College, for carrying out almost the whole of the experimental work.

#### SUMMARY.

1. Cereal plots were sampled by three different methods; two systematic, and one involving a random location of sampling units.

2. The disadvantages of the systematic methods as compared with random sampling, emerged clearly.

3. These disadvantages were further emphasised in an analysis of earlier data on sampling methods. For this purpose the methods and results of certain recent contributions to statistical theory were used.

4. By the use of a random sampling method, the variance due to sampling errors may be made a satisfactorily small fraction of the total variance of cereal plots one-fortieth of an acre in area.

#### REFERENCES.

- (1) ARNY, A. C. and GARBER, R. J. "Field Technic in Determining Yields of Plots of Grain by the Rod-Row Method." *J. Amer. Soc. Agron.* (1919) **11**.
- (2) ARNY, A. C. and STEINMETZ, F. H. "Field Technic in Determining Yields of Experimental Plots by the Square Yard Method." *J. Amer. Soc. Agron.* (1919) **11**.

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- (3) ENGLEADOW, F. L. "A Census of an Acre of Corn." *J. Agri. Sci.* (1926) **16**.
- (4) FISHER, R. A. *Statistical Methods for Research Workers*. Oliver and Boyd, Edinburgh (1925). 2nd edit. 1928.
- (5) FISHER, R. A. and MACKENZIE, W. A. "Studies in Crop Variation. II. The Manurial Response of Different Potato Varieties." *J. Agri. Sci.* (1923) **13**.
- (6) WISHART, J. "The Generalised Product Moment Distribution in Samples from a Normal Multivariate Population" *Biometrika* (1928) **20**.

### APPENDIX I.

#### TABLES OF PRIMARY DATA.

(The figures are in grams per metre: the grain figures in grams of dry weight per metre.)

#### I. BARLEY.

##### 1. Variety "824."

*Method (a). Random sampling.*

Block A		Block B		Block C	
Grain	Straw	Grain	Straw	Grain	Straw
28.47	61.3	56.18	90.5	22.56	34.3
78.83	101.7	64.04	95.2	41.99	62.3
93.87	137.9	45.96	62.6	14.45	37.9
35.40	58.1	55.17	88.7	28.90	50.8
53.31	51.9	69.62	127.6	33.04	50.9
55.34	73.5	36.08	79.3	27.88	63.0
65.99	35.9	54.07	84.0	41.99	53.3
44.61	72.2	35.74	78.7	49.09	81.9
61.42	86.3	47.82	92.4	45.37	63.3
29.40	50.2	50.53	90.2	51.45	73.1
546.64	729.0	515.21	889.2	356.72	570.8
Grand total		Grand mean			
Grain	Straw	Grain	Straw		
1418.57	2189.0	47.286	72.967		

*Method (b). Systematic sampling: symmetrical method.*

Set a		Set b		Set c		Set d		Set e		Set f	
Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
13.10	23.5	22.73	37.1	35.82	60.6	41.15	63.3	43.85	45.1	37.68	62.4
34.81	45.8	34.98	51.6	47.91	86.3	53.40	85.8	52.89	82.4	28.98	50.7
36.67	59.6	28.22	39.6	27.97	67.9	38.61	68.3	58.38	94.9	40.89	69.6
44.36	34.1	46.89	61.5	44.19	77.7	57.96	92.4	47.23	80.1	44.35	68.5
31.78	58.5	24.76	37.7	58.21	88.1	49.51	93.4	29.57	85.0	43.34	75.7
160.72	221.5	157.58	227.5	214.10	380.6	240.63	403.2	231.92	387.5	195.24	326.9
Grand total		Grand mean									
Grain	Straw	Grain	Straw			Grain	Straw			Grain	Straw
1200.19	1947.2	40.006	64.907								

## 2. Variety "Spratt Archer."

*Method (a). Random sampling.*

Block A		Block B		Block C	
Grain	Straw	Grain	Straw	Grain	Straw
63.84	122.4	80.94	117.8	62.01	86.7
62.40	108.2	47.12	70.5	43.41	65.2
34.06	69.0	78.57	109.8	42.05	61.9
41.73	80.3	37.30	39.9	51.40	73.1
35.24	64.5	61.38	82.5	35.16	55.6
48.95	79.2	68.67	109.3	51.40	68.1
30.49	61.5	79.75	119.3	34.14	54.9
85.30	162.3	57.50	64.4	57.66	81.2
48.55	69.7	34.45	95.5	36.51	50.9
23.68	65.1	48.39	40.9	35.80	62.8
474.24	882.2	594.07	849.9	449.54	660.4
Grand total		Grand mean			
Grain	Straw	Grain	Straw		
1517.85	2392.5	50.595	79.75		

*Method (b). Systematic sampling: symmetrical arrangement.*

Set a		Set b		Set c		Set d		Set e		Set f	
Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
63.87	74.4	27.80	27.1	51.71	68.8	52.97	85.3	59.14	70.0	48.24	59.9
19.86	25.5	47.90	51.3	53.82	63.3	40.89	56.6	46.55	55.9	36.16	64.2
34.81	45.8	29.82	39.7	60.66	66.2	48.58	57.5	57.96	85.4	83.39	99.3
44.10	102.8	47.48	51.8	45.79	58.8	62.61	49.9	63.11	77.3	49.93	61.9
32.95	49.0	53.23	62.0	29.99	34.5	53.74	71.4	52.97	71.3	77.31	96.5
195.59	297.5	206.23	231.9	241.97	291.6	258.79	320.7	279.73	359.9	295.03	381.8
Grand total		Grand mean									
Grain	Straw	Grain	Straw			Grain	Straw				
1477.34	1883.4	49.245	62.780								

## 3. Variety "Plumage Archer."

*Method (a). Random sampling.*

Block A		Block B		Block C	
Grain	Straw	Grain	Straw	Grain	Straw
21.15	34.3	75.70	109.4	45.62	56.0
49.34	77.7	37.94	48.1	32.28	31.8
74.53	118.9	49.93	61.9	8.79	11.6
76.03	124.0	56.10	67.6	22.56	29.3
93.77	167.6	62.35	87.2	23.83	33.8
54.33	90.4	67.25	91.4	16.14	22.9
21.62	28.7	62.10	86.5	36.67	40.6
65.97	119.7	31.35	48.9	48.07	60.1
43.87	85.6	41.57	56.8	42.08	48.2
23.21	53.7	38.36	35.6	39.71	50.0
523.82	900.6	522.65	693.4	315.75	384.3
Grand total		Grand mean			
Grain	Straw	Grain	Straw		
1362.22	1978.3	45.407	65.943		

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*Method (b). Systematic sampling: symmetrical method.*

Set a		Set b		Set c		Set d		Set e		Set f	
Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
45.79	53.8	37.26	37.9	41.15	50.3	33.46	56.4	43.77	58.2	28.30	55.5
22.98	37.8	22.64	38.2	71.99	90.8	34.98	32.6	56.61	62.0	24.25	50.3
31.77	35.4	29.32	42.3	75.87	102.2	51.79	71.7	36.67	55.6	14.28	20.1
27.54	38.4	30.75	44.6	83.90	112.7	67.59	91.0	32.53	44.5	6.17	9.7
22.31	22.6	34.39	44.3	42.58	53.6	37.60	53.5	58.04	71.3	29.66	44.9
150.39	188.0	154.36	207.3	315.49	409.6	225.42	305.2	227.62	291.6	102.66	180.5
Grand total						Grand mean					
Grain		Straw		Grain		Straw		Grain		Straw	
1175.94		1582.2		39.198		52.740					

### II. WHEAT.

*Variety "Red Standard."*

*Method (a). Random sampling.*

Block A		Block B		Block C	
Grain	Straw	Grain	Straw	Grain	Straw
26.50	66.1	36.98	107.7	47.85	154.8
37.14	91.5	37.29	111.3	120.10	344.4
30.49	74.0	42.14	139.1	51.06	147.7
37.14	105.5	34.56	87.8	45.74	125.5
52.39	129.0	35.11	88.1	47.23	110.6
36.04	93.9	41.44	110.0	17.98	62.0
48.01	181.6	37.37	73.2	24.39	64.8
20.33	61.0	46.52	109.5	58.95	143.6
12.12	54.5	25.33	65.6	36.83	103.9
28.07	102.1	37.22	73.4	48.87	115.5
328.23	959.2	373.96	965.7	499.00	1372.8
Grand total		Grand mean			
Grain		Straw		Grain	
1201.19		3297.7		40.040	
				109.923	

*Method (b). Systematic sampling: symmetrical method.*

Set a		Set b		Set c		Set d		Set e		Set f	
Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
41.99	88.3	27.52	60.8	38.70	76.5	20.09	47.3	47.38	112.4	29.40	79.4
23.30	51.2	40.66	85.0	46.29	96.8	34.25	79.2	41.28	114.2	45.43	76.9
42.22	92.0	31.67	67.5	30.49	74.0	41.12	100.4	32.37	88.6	34.18	87.3
26.89	65.6	35.18	79.0	60.52	137.6	42.30	94.9	46.60	111.4	36.20	93.7
40.50	83.2	30.81	68.6	88.75	217.5	29.40	66.4	41.83	123.5	33.70	93.9
174.90	380.3	165.84	360.9	264.75	602.4	167.16	388.2	209.46	550.1	178.91	431.2
Grand total						Grand mean					
Grain		Straw		Grain		Straw		Grain		Straw	
1161.02		2713.1		38.701		90.473					

*Method (c). Random row method.*

Row 1		Row 3		Row 19		Row 20		Row 24	
Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
94.58	184.9	22.08	60.3	30.49	69.5	32.13	163.6	9.53	25.9
90.02	221.2	33.93	80.5	36.59	104.4	29.03	68.2	36.34	96.7
86.41	195.4	33.93	67.5	30.24	61.8	48.88	94.1	42.18	94.9
114.93	267.2	30.24	73.8	50.42	93.3	24.91	110.0	51.02	98.6
148.95	311.6	46.99	121.3	46.39	165.0	51.20	127.4	60.99	136.0
93.55	221.1	30.07	76.0	50.59	133.1	30.07	96.0	43.29	112.6
628.44	1401.4	197.24	479.4	244.72	627.1	216.22	659.3	243.35	564.7
Grand total				Grand mean					
Grain		Straw		Grain		Straw			
1529.77		3731.9		50.992		124.397			

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# A STUDY IN SAMPLING TECHNIQUE: THE EFFECT OF ARTIFICIAL FERTILISERS ON THE YIELD OF POTATOES.

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(With One Text-figure.)

## INTRODUCTION.

IN order to test the adequacy of a sampling method for estimating the yield of a crop of potatoes, use was made of the plots of a part of the Rothamsted Potato Experiment of 1928. This experiment was designed to give information as to the effect on yield of applying nitrogenous, potassic and phosphatic fertilisers in various quantities. There was a basal dressing of dung at the rate of 14 tons per acre, and further nitrogen was supplied as sulphate of ammonia at rates of 0,  $1\frac{1}{2}$  and 3 cwt. per acre; potash at rates equivalent to 0, 1 and 2 cwt. of sulphate of potash per acre; and phosphate as superphosphate at 3 cwt. per acre. The experiment was of the "Randomised Blocks" pattern devised by R. A. Fisher(1), and consisted of 81 plots arranged in 9 blocks. The effect of phosphate was found by dividing each plot into two equal sub-plots, only one of which, chosen at random, received superphosphate. The arrangement is shown in the figure.

The variety was "Ally." The sets were planted on April 17th-19th, and the crop lifted on October 19th. Each of the 162 sub-plots was  $\frac{1}{90}$ th of an acre in area, consisted of three bouts 3.4 links apart and 108 links long, and included about 180 plants.

Three blocks of the experiment (*A*, *B* and *C* of the figure), comprising 54 sub-plots in all, were first sampled by the method described below, and then lifted with a Howard's "Spinner," followed by harrows, and weighed on the field, in order to find the total yield from each sub-plot.

## THE STATISTICAL PROBLEM.

It was pointed out in an earlier paper(2) that a valid estimate of error of sampling can only be made if the constituent parts of a sample are located independently and at random. These constituent parts need not necessarily be individual plants or single short lengths of drill, but

may be complex patterns as in the "échelon" method used by Engledow (5), provided that a random selection can be made from a "population" of patterns.

To avoid confusion the following terms will be used in the strictly technical sense indicated:

(a) "*Units*"—the ultimate parts of a sample. For cereals, short lengths of drill, or small areas; for roots and potatoes, individual plants, etc.

A			B			C		
3 O	6 P	9 O	9 P	6 O	5 O	2 O	9 P	4 O
3 P	6 O	9 P	9 O	6 O	5 P	2 P	9 O	4 P
1 O	7 O	2 O	8 O	4 O	1 O	7 O	8 P	5 P
1 P	7 P	2 P	8 P	4 P	1 P	7 P	8 O	5 O
4 O	8 P	5 O	7 O	2 P	3 O	1 P	3 O	6 P
4 P	8 O	5 P	7 P	2 O	3 P	1 O	3 P	6 O
2 P	7 P	8 O	5 O	9 P	6 O	7 P	2 O	4 P
2 O	7 O	8 P	5 P	9 O	6 P	7 O	2 P	4 O
3 O	6 O	5 O	1 O	4 O	7 O	5 P	8 P	9 O
3 P	6 P	5 P	1 P	4 P	7 P	5 O	8 O	9 P
4 O	9 O	1 O	2 O	3 O	8 P	3 P	1 P	6 P
4 P	9 P	1 P	2 P	3 P	8 O	3 O	1 O	6 O
9 O	6 P	7 P	3 P	9 P	8 P	2 O	6 O	1 P
9 P	6 O	7 O	3 O	9 O	8 O	2 P	6 P	1 O
2 O	8 P	4 P	4 O	1 O	7 P	3 O	5 O	8 O
2 P	8 O	4 O	4 P	1 P	7 O	3 P	5 P	8 P
1 O	3 P	5 P	2 P	6 P	5 O	4 O	9 O	7 O
1 P	3 O	5 O	2 O	6 O	5 P	4 P	9 P	7 P
G			H			I		

Key to treatments		
No.	Sulphate of ammonia cwt. per acre	Sulphate of potash cwt. per acre
1	0	0
2	1½	0
3	3	0
4	0	1
5	1½	1
6	3	1
7	0	2
8	1½	2
9	3	2

P—3 cwt. superphosphate per acre.

O—No superphosphate.

Fig. 1.

(b) "*Sampling-units*"—those parts of a sample which are located independently and at random within the area to be sampled. Each may consist of one or many "units."

(c) "*Sample*"—the aggregate of sampling-units taken from the area. A valid estimate of error is only obtainable if each sample consists of at least two sampling-units.

The interrelations of these parts determine three important characteristics of the sampling-error:

(a) *Validity*—at least two sampling-units, defined as above, are

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necessary to provide a valid estimate of error. The number and distribution of units within the sampling-units play no part whatever in determining the validity of the estimate.

(b) *Magnitude*—*i.e.* accuracy of the estimate of yield—the magnitude of the error may be reduced either by increasing the number of sampling-units in the samples, or by adjusting the number and distribution of units within the sampling-unit. Care in spacing the units within large sampling-units will tend to make the latter individually representative of the area sampled, and therefore like one another—*i.e.* the sampling-error will be small. There is, however, a further point to be considered—the units should be located in such a way as to minimise the actual labour of sampling. This is of considerable importance where many plots have to be sampled, and provides a strong case for a simple systematic distribution of units.

(c) *Accuracy*—*i.e.* accuracy of the estimate of sampling-error—the relative accuracy is determined solely by the number of degrees of freedom on which the estimate is based; but, of course, the standard error of an estimate of the variance or standard deviation is proportional to the absolute magnitude of the value to be estimated.

Since the observations were made on a whole set of experimental plots (54), they differed essentially from those on cereals(2), where individual plots were dealt with. For assessing the yield of a single plot it is necessary to take a fairly large number of sampling-units, the actual number, of course, depending on the size of the plot and the uniformity of the crop, but rarely being less than 10. If fewer are taken the estimate of error is too inaccurate to be of much value. When a large number of plots is being sampled, however, as in the present case, each plot may be made to contribute to the estimate of error, if it can be assumed that the variation from sample to sample is sensibly the same on different plots. There is a considerable body of agricultural data to justify this assumption for plots bearing the usual English crops manured according to current practice, though it is certainly not true over the large range of manurial treatment given in pot-experiments, where the heaviest application may be twenty or thirty times the lightest. The experiment under notice being a field trial in which additional nitrogen was given at rates not exceeding 0.6 cwt. per acre, potash and phosphate being given at still smaller rates, it was satisfactory to assume that the variance was constant over all treatments. Under the circumstances it became possible to sample only in duplicate—*i.e.* to take only two sampling-units from each of the 54 sub-plots, the sampling-unit being a complex

structure designed to be highly representative of the plot, yet easily and rapidly taken.

Now we are dealing with a crop in which the individual plants are quite large and at some distance apart. The considerable variability usually present in the spacing of the plants makes a small metrical unit undesirable: greater lengths of bout would give unduly large units. The individual plant thus becomes the logical unit.

In the present case duplicate sampling-units were taken of every twentieth plant on each plot, the starting-points being selected at random from among the first twenty plants. It is easy to see that there are 190 different ways of choosing a pair of sampling-units from any one plot, so that if there are  $n$  plots, the method envisages a population of  $190n$  pairs of sampling-units from which  $n$  are chosen at random. The conditions for a valid estimate of error are therefore fulfilled. Further, since there were 54 sub-plots, a direct estimate is based on  $54 \times 1 = 54$  degrees of freedom, and is therefore well-founded.

#### THE FIELD TECHNIQUE OF SAMPLING.

The field technique of sampling was as follows: from a note-book containing a list of pairs of "starting-points" chosen at random was taken the pair of numbers which had been assigned to the sub-plot about to be sampled, and a white wooden peg was stuck in the ground beside the plant corresponding to the smaller of the two numbers. Similar white pegs marked every twentieth plant from this starting-point. The total number of pegs was noted, and also the number of plants beyond the last peg. This gave sufficient data for calculating the number of plants in the sub-plot. A black peg was then placed beside the plant corresponding to the larger of the two numbers, and at every twentieth plant from it. There were thus two sets of pegs marking the constituents of the two sampling-units to be taken from the sub-plot.

Each marked plant was lifted with a fork, care being taken not to disturb neighbouring plants, and the tubers rubbed to remove some of the adhering soil. They were then put in a basket and taken to the balance (placed on a table in the middle of the plot) where their total weight was found to the nearest ounce. The peg marking the plant was placed in the basket with the tubers, so that there was little danger of entering the weight wrongly in a note-book whose pages were divided into two columns, one for "black" and one for "white" sampling-units.

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### DETERMINATION OF THE SAMPLING-ERROR.

For the two sampling-units selected and weighed on each plot the following calculations were performed: (*a*) and (*b*) mean weight of a plant in ounces; (*c*) difference between mean plant-weights for duplicate sampling-units, *i.e.* (*a*)  $\sim$  (*b*); (*d*) and (*e*) sum of squares (in ounces<sup>2</sup>) of the deviations of individual plant-weights from the mean of the sampling-unit, and (*f*) the sum (*d*) + (*e*). A specimen page from the note-book is set out herewith, the plot selected being the half of B4 treated with superphosphate.

Table I.

	Weight in oz.	
	1st sampling-unit	2nd sampling-unit
Plot B 4 P	23	18
	16	15
	19	17
	17	22
	20	22
	12	18
	12	25
	18	31
	23	
Number of plants ...	9	8
Mean weight in oz. ...	17.8	21.0
Difference ( <i>c</i> ) ...	3.2	
Sum of squares ...	132	188
Sum ( <i>f</i> ) ...	320	

Actually 52 plots out of the 54 in Blocks A, B and C were dealt with in this way. The remaining two plots were left out of the calculation owing to uncertainty as to whether certain plants had been correctly assigned to sampling-units 1 and 2 respectively. The mean plant-weight over all the 52 plots was 19.104 oz. The sum of squares of deviations of the means of duplicate sampling-units from their common mean is, for this plot,  $\frac{1}{2} (3.2)^2$ , with 1 degree of freedom. Leaving the factor  $\frac{1}{2}$  to be introduced at a later stage, the total sum of squares of deviations between actual means is obtained by adding together the squares of all these differences (*c*), one for each plot. The total is 757, with 52 degrees of freedom. Dividing this total by 52 and then by 4, and taking the square root, we obtain the standard error of the mean of duplicate sampling-units. But in order to see if the complex sampling-unit used is appreciably better—*i.e.* more representative of the plot—than a sample of the same number of independently located units, we may also calculate the variance between the means of samples selected at random,

in the following way. Sum all the quantities ( $f$ ), *i.e.* find the total sum of squares of deviations of each plant-weight from the mean of the sampling-unit in which it is contained. This amounts to 45,921. Divide by the number of degrees of freedom (one less than the number of plants for each sampling-unit). Grouping according to the number of plants we have

No. of plants	Frequency	Degrees of freedom
7	2	12
8	43	301
9	55	440
10	4	36
	<hr/> 104	<hr/> 789

On dividing 45,921 by 789 we are left with 58·2015. This is the variance between plants. That between random means, for comparison with the figure 14·558 obtained for the variance between actual means, is obtained by multiplying 58·2015 by

$$\frac{2}{104} \times \left\{ \frac{2}{7} + \frac{43}{8} + \frac{55}{9} + \frac{4}{10} \right\},$$

*i.e.* by 1/4·27216. The following table shows the results reached up to this point.

Table II.

	Degrees of freedom	Sum of squares	Mean square
Between plants ...	789	45,921	58·202
Between random means ...	789	10,749	13·623
Between actual means ...	52	757	14·558
	<hr/> 841	<hr/> 11,506	<hr/> 13·681

The variances between actual and random means are not significantly different, so that there is no apparent gain by the systematic arrangement, and we may take the combined figure 13·681 as the best estimate of sampling variance. Dividing by 4 and taking the square root of the result, we find that the standard error of a 1 in 10 sample is 1·8494, or 9·68 per cent. of the mean plant-weight 19·104 oz.

Table III.

	Degrees of freedom	Sum of squares	Mean square
Between plants ...	1133	205,807	181·648
Between random means ...	1133	24,642·4	21·750
Between actual means ...	36	507·2	14·089
Mean plant weight = 37·086 oz.			

Standard error of sampling =  $\sqrt{\{14·089 \div 4\}} = 1·877$ , or 5·06 per cent.

It is interesting to compare the figure 9·68 with the analogous figure obtained from an experiment carried out at Woburn. Two blocks of

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eighteen plots each were here selected for testing the sampling method, and the above results (Table III) obtained in precisely the same way as has been described. An interesting feature of these results is that the systematic arrangement has shown itself definitely superior to purely random sampling, as is seen on comparing the variance "between random means" with that "between actual means":

	Variance	$\frac{1}{2} \log_e (\text{variance})$
Between random means	21.750	1.5398
Between actual means	14.089	1.3227

The difference between half the natural logarithms of the variances is 0.2171. Looking up R. A. Fisher's table of  $z(3)$  with  $n_1 = 1133$  and  $n_2 = 36$ , we find that we are just about at the 5 per cent. significance level, *i.e.* only once in twenty times would such a difference occur in two sample estimates of the same variance.

In view of the significance of this difference the smaller value, 14.089, is used in calculating the sampling-error, which is then found to be 5.06 per cent. This result may be interpreted as showing that the Woburn soil was more heterogeneous within plots than was the soil at Rothamsted, where there was no difference between the variances, and where, consequently, an average value was used.

The two estimates of error, 9.68 per cent. at Rothamsted, and 5.06 per cent. at Woburn, are not comparable figures, in that the former is based on plots only 1/90th acre in area, while at Woburn the plots were 1/40th acre in area. Since, however, the sub-plots at Rothamsted were arranged in pairs whose members were similarly treated except that only one received superphosphate, it is possible to arrive at an estimate of sampling-errors over plots of 1/45th acre, an area very nearly that of the Woburn plots. This is effected as follows: the differences in mean root-weight between members of the adjacent pairs of sub-plots are found for each pair, and from these are calculated the mean difference due to superphosphate, and also the sum of the squares of deviations from this mean difference. This latter quantity may fairly be taken as arising from soil differences between adjacent sub-plots. (It would also include interaction between phosphate and other manurial treatments, but these are shown to be insignificant.)

The variance of duplicate sampling-units from the same sub-plot provides an estimate of errors due to sampling alone. If now we add to the corresponding sum of squares that due to soil-difference between adjacent sub-plots (both reduced to a sub-plot basis), we can arrive at an estimate of the variance of a similar sample distributed over the

double area. Halving the figure gives the variance of a sample twice as large, and from the double area—1/45th acre.

Table IV.

	Degrees of freedom	Sum of squares	Mean square	Standard error
Differences due to sampling within sub-plots ... ..	841	2876.5	3.420	—
Soil differences between sub-plots	26	161.44	6.209	—
Total ... ..	867	3037.9	3.5039	—
			1.7520	1.324 or 6.93 %

It is noteworthy that plant number contributes a very much larger share to the total variation than does soil-heterogeneity. For on the whole plot of 1/45th acre the sampling variance of a 1 in 10 sample is little more than half that on 1/90th acre. (Doubling the plant number, if there were no soil effects, should halve it exactly.) This being so it is possible to estimate the corresponding figure for 1/40th acre, by neglecting the soil-factor. The variance, 1.7520, must now be multiplied by 8/9, giving 1.577 as the new figure. Hence the standard error becomes:

$$\sqrt{1.577} = 1.248, \text{ or } 6.53 \text{ per cent.}$$

This figure is now directly comparable with the Woburn result of 5.06 per cent. Comparing the variances in units of 1 per cent.:

At Rothamsted  $6.53^2$  or 42.64,

At Woburn  $5.06^2$  or 25.60,

it is seen that the sampling is considerably more accurate at Woburn than at Rothamsted. This is probably due to the much greater percentage at Rothamsted of plants suffering from Leaf Roll, leading to a bigger variation in root-weight from plant to plant.

#### STATISTICAL TREATMENT OF ACTUAL AND ESTIMATED YIELD FIGURES.

Table V.

Actual yields in half lb.

A			B			C		
1	2	3	1	2	3	1	2	3
391	521	480	531	564	454	503	563	494
401	402	493	449	512	509	459	526	491
4	5	6	4	5	6	4	5	6
284	299	395	384	361	337	365	459	453
278	349	438	427	365	395	420	460	502
7	8	9	7	8	9	7	8	9
283	426	411	318	410	420	312	383	565
290	370	387	286	360	412	289	420	543
Block totals	6898			7494			8207	

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The total produce from each plot was weighed and the weight of the samples previously taken from the plots added in, the total being recorded to the nearest half pound. Table V shows the serial numbering of the plots, for comparison with Fig. 1, and the actual yields in half pounds.

Table VI.  
Treatment totals in half lb.  
Amount of S/Amm. in cwt. per acre

Totals of 3 plots		0	1½	3	Total	
Amount of potash in cwt. per acre S/Pot.	0	(P 985	1307	1233	3,525	
		(O 910	1258	1194	3,362	
	1	(P 1146	1349	1650	4,145	
		(O 1138	1367	1457	3,962	
	2	(P 1055	1312	1587	3,954	
		(O 982	1214	1455	3,651	
	Total ...	(P 3186	3968	4470	11,624	Phosphate total
		(O 3030	3839	4106	10,975	No phosphate total
Grand total ...		22,599				General mean ... 418.5

## ANALYSIS OF VARIANCE. AGRICULTURAL YIELDS.

The 53 degrees of freedom may be analysed in the first instance into 2 for differences between different blocks of land, 17 for differences between treatments, and 34 on which to base an estimate of error. Of the 17 degrees of freedom 8 are accounted for by the nitrogenous and potassic treatments, and are obtained (see Table VI) from the nine totals of adjoining phosphate and no phosphate sub-plots (2 for nitrogen, 2 for potash and 4 for the interaction of these fertilisers). We then find that 16 ( $8 \times 2$ ) degrees of freedom out of the 34 for error are appropriate for furnishing an estimate of the error of the nitrogen-potash comparisons, while the remaining 18 are to be used for the phosphate comparison. The remaining 9 degrees of freedom for treatment consist of 1 for the direct comparison phosphate *versus* no phosphate, and 8 for the phosphate interactions with nitrogen and potash. The analysis is as follows:

Table VII.

	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2} \log_e$ (mean square)
Blocks ... ..	2	47,723.44	23,861.72	—
Nitrogen ... ..	2	160,967.44	80,483.72	5.6479
Potash ... ..	2	41,776.44	20,888.22	4.9735
Interaction ... ..	4	21,432.78	5,358.20	4.2932
Error (a) ... ..	16	49,662.89	3,103.93	4.0202
Phosphate ... ..	1	7,800.02	7,800.02	4.4809
Phosphate interactions ...	8	5,456.82	682.10	3.2626
Error (b) ... ..	18	14,227.67	790.43	3.3363
Total ... ..	53	349,047.50	—	—

It will be noticed that the error for the phosphate comparison is significantly smaller than the other, as we have a right to expect, for the treatments compared were on two adjacent plots throughout, and soil-differences would be much smaller than between plots separated by larger distances. In fact the correlation between adjacent sub-plots was + 0.59. Now examining the last column of the table, where half the natural logarithm of the variance is given, we find from the table of  $z$ , (i) that the effect of nitrogen and of potash is undoubtedly significant—not once in a hundred times would such a large difference occur in two sample estimates of the same variance—while the interaction of potash and nitrogen is not significant, (ii) that phosphate shows an undoubtedly significant effect—the probability of a difference of 1.1446 is less than 0.01—while the phosphate interactions are not significant.

We may therefore present our results in the form of two tables, one showing the straight comparison between the plots receiving and not receiving phosphate, *i.e.* between the means of 27 sub-plots, while the other shows the nitrogen and potash effects, and is obtained by averaging in each case the two sub-plots shown in Table VI. The standard errors appropriate to these comparisons are:

*Phosphate comparison.*

$$\begin{aligned}\text{Standard error (mean of 27 plots)} &= \sqrt{\{790.43 \div 27\}} \\ &= 5.41 \text{ half lb.};\end{aligned}$$

or 1.29 per cent. of the mean yield 418.5 of the sub-plot.

*Nitrogen and potash comparisons.*

$$\begin{aligned}\text{Standard error (mean of 6 plots)} &= \sqrt{\{6207.86 \div 6\}} \\ &= 32.17 \text{ half lb.};\end{aligned}$$

or 3.84 per cent. of the mean yield 837 of the whole plot.

Expressed in tons per acre, and as a percentage of the mean yield, the results are as follows:

Table VIII. *Summary of results—Actual yields.*

Average yield		Without superphosphate	With superphosphate	Mean	Standard error
Tons per acre ...		8.17	8.65	8.41	0.11
Per cent. ...		97.1	102.9	100.0	1.29

Average yield in tons per acre				Per cent.				
S/Amm.				S/Amm.				
				0	1½	3		
Potash	{	0	6.34	8.59	8.13	75.5	102.2	96.7
		1	7.65	9.09	10.40	91.0	108.2	123.7
		2	6.82	8.46	10.19	81.1	100.6	121.1
		Standard error ...		0.32	Standard error ...		3.84	

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The principal conclusions from the experiment are: There is a significant response to the nitrogenous, potassic and phosphatic fertilisers. The response to phosphate was small, but was detected by the precise nature of the comparison made possible by the design of the experiment. There was no further response to the double dressing (2 cwt.) of potash, or to the double dressing (3 cwt.) of sulphate of ammonia in the absence of potash.

### ESTIMATED YIELDS FROM SAMPLING.

If the total produce had not been weighed, the yields would have had to be estimated by multiplying the mean plant-weight of the samples taken on any one plot by the total number of plants on that plot. The results, for comparison with Table V, are given below:

Table IX.

Estimated yields in half lb.

A			B			C		
1	2	3	1	2	3	1	2	3
336	564	558	505	631	427	557	506	461
369	399	565	440	579	500	508	528	487
4	5	6	4	5	6	4	5	6
253	292	327	341	437	337	372	386	360
167	348	426	405	284	392	407	366	575
7	8	9	7	8	9	7	8	9
268	360	346	301	394	420	282	320	603
251	345	400	286	366	449	286	426	549
Block totals			6574			7494		
						8039		

While the variations of the estimated and actual yields for individual plots are in some cases large, the treatment and block averages agree very well. It is a striking coincidence that the totals for Block B should be identical. The treatment totals are as follows:

Table X.

Estimated treatment totals in half lb.

Amount of S/Amm. in cwt. per acre

Totals of 3 plots		0			1½			3			Total	
Amount of potash in cwt. per acre S/Pot.	0	{	P	841	1328	1244	3,413					
			O	876	1250	1076	3,202					
	1	{	P	1022	1260	1798	4,080					
			O	1166	1348	1527	4,041					
	2	{	P	1041	1151	1636	3,828					
			O	965	1052	1526	3,543					
Total ...	{	P	2904	3739	4678	11,321	Phosphate total					
		O	3007	3650	4129	10,786	No phosphate total					
Grand total ...				22,107				General mean ...		409.38		

There is a slight underestimate of the total crop yield, the mean 409.39 half lb. corresponding to a yield of 8.22 tons per acre, as compared with 8.41 tons per acre actual yield.

## ANALYSIS OF VARIANCE. ESTIMATED YIELDS.

Table XI.

	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2} \log_e$ (mean square)
Blocks ... ..	2	60,919.44	30,459.72	—
Nitrogen ... ..	2	233,000.44	116,500.22	5.8328
Potash ... ..	2	63,001.33	31,500.66	5.1789
Interaction ... ..	4	69,753.90	17,438.48	4.8832
Error (a) ... ..	16	95,762.22	5,985.14	4.3485
Phosphate ... ..	1	5,300.46	5,300.46	4.2878
Phosphate interactions ...	8	22,221.37	2,777.67	—
Error (b) ... ..	18	54,129.67	3,007.20	4.0044
Total ... ..	53	604,088.83	—	—

Table XII. *Summary of results—Estimated yields.*

Average yield		Without superphosphate	With superphosphate	Mean	Standard error		
Tons per acre ...		8.03	8.42	8.22	0.21		
Per cent. ...		97.6	102.4	100.0	2.58		
Average yield in tons per acre S/Amm.				Per cent. S/Amm.			
		0	1½	3	0	1½	3
Potash	0	5.75	8.63	7.77	69.9	105.0	94.4
	1	7.33	8.73	11.13	89.1	106.2	135.4
	2	6.72	7.38	10.59	81.7	89.7	128.7
Standard error ...		0.45		Standard error ...		5.46	

Nitrogen and potash still show significant responses, while the variance due to interaction of these two fertilisers is larger than before, but does not quite approach the 5 per cent. level of significance. The phosphate effect, however, is insignificant. There is evidence from the agricultural yields that it exists, although only to a moderate extent. The larger standard error on the estimated yields, which necessarily includes the error due to sampling, has masked what was at best only a moderate response to the phosphatic dressing.

The standard errors are:

*Phosphate comparison.*

$$\begin{aligned} \text{Standard error (mean of 27 plots)} &= \sqrt{\{3007.2 \div 27\}} \\ &= 10.55 \text{ half lb.;} \end{aligned}$$

or 2.58 per cent. of the mean yield 409.4 of the sub-plot.

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*Nitrogen and potash comparisons.*

$$\text{Standard error (mean of 6 plots)} = \sqrt{\{11970 \cdot 28 \div 6\}} \\ = 44 \cdot 67 \text{ half lb.};$$

or 5·46 per cent. of the mean yield 818·8 of the whole plot.

The standard error appropriate to the phosphate comparisons is rather less than one-half that for the potash and nitrogen comparisons, as compared with one-third in the case of the actual yield figures. This indicates that there is some factor working against the favourable circumstance of the plots to be compared for the phosphatic effect being contiguous. The accuracy of the sampling method depends on the proportion of plants actually selected for weighing. This could be increased, but a point would ultimately be reached where there would be no substantial gain, either in time or economy of working, over the ordinary method of harvesting the whole crop. But given a certain sampling ratio, as the 1 in 10 of this experiment, the accuracy may be affected by differences in the numbers of plants on the several plots, or by an uneven crop. This field was admittedly patchy, the weight of tubers from individual plants sampled varying from 2 to 53 oz. The least satisfactory feature of the analysis conducted on the basis of the estimated yield figures from the samples is the obscuring of the effect of the phosphatic fertiliser by an increased standard error. To see how far this effect is due to variation in individual plant-weight, and how far to variation in plant-number from plot to plot, a corresponding calculation was made on the sampling plant-weight, *i.e.* the mean weight in oz. of a plant, estimated from duplicate sampling-units from the same sub-plot. Set out in half oz. units the figures are as follows:

Table XIII.

Sampling plant-weights in half oz.

A			B			C		
1	2	3	1	2	3	1	2	3
32·6	51·3	53·1	46·2	58·7	40·9	52·1	53·6	43·4
34·7	36·5	55·5	41·9	53·2	47·1	48·1	50·3	46·7
4	5	6	4	5	6	4	5	6
23·4	27·6	32·1	31·2	40·4	31·5	35·6	35·9	33·1
16·8	34·8	40·1	38·8	27·4	37·1	40·4	34·2	54·1
7	8	9	7	8	9	7	8	9
24·8	34·1	33·0	29·2	38·0	40·0	28·6	30·5	58·1
23·6	31·5	37·9	27·9	35·1	41·8	26·9	37·9	48·8
Block totals	623·4		706·4			758·3		

The actual number of plants per sub-plot may be obtained by dividing the figures in Table IX by those in Table XIII and multiplying

by 16. The numbers are fairly regular, varying from 158 to 180. In fact a cursory inspection of the two tables shows that the more important divergences from the actual yields of Table V are reflected in both, and are due, therefore, to the samples being in some cases insufficiently representative of the growing crop.

Table XIV.

		Treatment totals in half oz. Amount of S/Amm. in cwt. per acre				
Totals of 3 plots		0	1½	3	Total	
Amount of potash in cwt. per acre S/Pot.	0	{ P	82.5	126.2	114.4	323.1
		{ O	81.8	119.3	103.1	304.2
	1	{ P	97.7	118.1	168.1	383.9
		{ O	108.6	128.0	138.5	375.1
	2	{ P	103.1	108.8	155.3	367.2
		{ O	92.4	96.9	145.3	334.6
	Total ...	{ P	283.3	353.1	437.8	1074.2
		{ O	282.8	344.2	386.9	1013.9
	Grand total ...		2088.1			38.6685
					General mean ...	

## ANALYSIS OF VARIANCE. SAMPLING PLANT-WEIGHT.

Table XV.

		Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2} \log_e$ (mean square)
Blocks ...	...	2	514.46	257.23	—
Nitrogen ...	...	2	1857.74	928.87	3.4170
Potash ...	...	2	484.57	242.28	2.7450
Interaction ...	...	4	679.38	169.84	2.5674
Error (a) ...	...	16	913.25	57.08	2.0222
Phosphate ...	...	1	67.34	67.34	2.1049
Phosphate interactions ...	...	8	203.47	25.43	—
Error (b) ...	...	18	443.35	24.63	1.6020
Total ...	...	53	5163.56	—	—

The effect of the nitrogenous and potassic fertilisers shows up in very much the same way as it did in the analysis of the estimated yield figures, while that due to phosphate, though still not significant, is distinctly larger than before. It is interesting to note that the difference between the errors (a) and (b) is again significant, although not to the same marked extent as in the case of the actual yield analysis. This shows that the variation in plant-number between contiguous sub-plots was so large as partially to obscure the advantages known to follow from the arrangement.

The standard errors are:

*Phosphate comparison.*

Standard error (mean of 27 plots) =  $\sqrt{24.63 \div 27}$   
 — 0.96 half oz.;

or 2.47 per cent. of the mean yield 38.67 of the sub-plot.

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*Nitrogen and potash comparisons.*

Standard error (mean of 6 plots) =  $\sqrt{\{114.16 \div 6\}}$

= 4.36 half oz.;

or 5.64 per cent. of the mean yield 77.34 of the whole plot.

Table XVI. *Summary of results—Mean sampling plant-weight.*

Average yield		Without superphosphate	With superphosphate	Mean	Standard error		
Oz. per plant	...	18.78	19.89	19.33	0.48		
Per cent. ...	...	97.1	102.9	100.0	2.47		
Average yield in oz. per plant S/Amm.				Per cent. S/Amm.			
<div> <div>0</div> <div>1½</div> <div>3</div> </div>				<div> <div>0</div> <div>1½</div> <div>3</div> </div>			
Potash	0	13.69	20.46	18.12	70.8	105.8	93.7
	1	17.19	20.51	25.55	88.9	106.1	132.1
	2	16.29	17.14	25.05	84.3	88.7	129.6
Standard error ...		1.09		Standard error ...		5.64	

On taking these results in conjunction with the earlier ones, it is apparent that the phosphate effect has been rendered less definite than it was in reality from two causes (*a*) the error of sampling, and (*b*) the variation in plant-number from plot to plot. These causes have acted together and in the same direction; they have reduced the actual difference between the means of the phosphate and no-phosphate sub-plots, and at the same time have increased the standard error of this difference. Apart from this, the sampling method may be held to have been very successful in showing up the main fertiliser effects at only a fraction of the labour and cost necessarily incurred in the complete harvesting and separate weighing of all the plots.

### DISCUSSION.

The comparison of errors due to soil differences and experimental errors, with those due to sampling, is best made by the calculation of relative variances  $\left(\frac{\text{variance}}{(\text{mean})^2}\right)$  per plot.

Table XVII.

Relative variance  $\times 10^4$ .

				Rothamsted		Woburn
				Whole plots	Half- plots	
Agricultural yields	...	...	...	88.6	45.1	50.4
Sampling relative variance	...	...	...	46.8	93.7	25.6

The sampling variance is unsatisfactorily high as compared with the variance due to other causes, especially in the case of half-plots, where

the fact that adjacent half-plots are considered reduces the variance due to soil differences very considerably. That the high values at Rothamsted are due to some extent to the severe infection with Leaf Roll is borne out by examination of the corresponding figures for Woburn, where, however, the plots were of  $1/40$ th acre. It appears that a 1 in 10 sample is definitely inadequate for plots of  $1/90$ th acre: had the sampling method alone been relied upon, the effect of phosphate would have appeared insignificant. It is somewhat unsatisfactory, too, for plots of  $1/45$ th or  $1/40$ th acre, for even at Woburn the sampling variance was half as great as the agricultural-yield variance. It must in fact be considered fortunate that all the treatment differences for whole plots appeared significant on analysing the sampling-yields in the Rothamsted experiment.

It is easy to estimate from the analyses how many plants it would be necessary to lift from each plot in order to give a sampling-error of, say, 4 per cent. At Rothamsted the number is 102, and at Woburn 56. A sample consisting of this number of plants would raise a standard error per plot from 7 per cent. only to 8 per cent., and the reduced labour of lifting might compensate for the slight loss of accuracy. At Rothamsted it would be necessary to take every third or fourth plant of a plot of  $1/40$ th acre in order to reduce the sampling-error to this extent, while for plots of  $1/20$ th acre every seventh plant would be needed. At Woburn, the error being smaller, every sixth and every twelfth plant respectively would have been sufficient. Little would be gained, then, by the use of a sampling method for small plots. With large plots, of  $1/20$ th acre or more in area, however, the labour costs of lifting and weighing the crop would be very considerably diminished without prejudicing the value of an experiment.

A comparison of the relative variances per plot, derived on the one hand from the agricultural yields and on the other from the sampling yields, should provide an indirect estimate of errors due to the sampling technique. For in the case of the agricultural yields, the plot variance arises from differences in soil-fertility on different plots; from inaccuracies in area measurements and weighings; and from the fact that there is a comparatively small number of plants on a plot. The sampling-yields are subject to all these errors, but to the last in a greater degree, since only one-tenth of the total number of plants is weighed. The differences between the two plot variances, expressed in suitable units, should therefore be an estimate of nine-tenths of the variance due to sampling.

The accompanying table shows the relative variances for whole plots

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and for half-plots, arising from factors other than block and treatment differences.

Table XVIII.

Relative variance  $\times 10^4$ .

			Whole plots	Half-plots
Agricultural yields	...	...	88.6	45.1
Sampling yields	...	...	178.6	179.4
Difference $\times 10/9$	...	...	99.9}	149.2}
Sampling variance	...	...	46.8}	93.7}

The quantities enclosed in brackets should be equal. They appear to differ considerably: the indirect estimate is about double the direct estimate. The reason for the apparent discrepancy is the very high variance of the indirect estimate, based as it is on the difference between two variances. Thus the variance of the difference between two variances  $v_1$  and  $v_2$ , based on  $n$  degrees of freedom, is given by the formula

$$V_{v_1-v_2} = 2(v_1^2 + v_2^2 - 2r_{v_1 v_2} v_1 v_2)/n,$$

where  $r_{v_1 v_2}$  is the correlation between the variances in samples.  $r_{v_1 v_2}$  has been shown (4) to be exactly equal to  $\rho_{12}^2$ , where  $\rho_{12}$  is the population value of the correlation between the two variates. As the best available estimate of this parameter we may use  $r_{12}$ , the value observed in samples. In the present case  $r_{12}$  is the "remainder" correlation between the corresponding values of the agricultural and sampling yields.

We have, then,

$$V_{v_1-v_2} = 2(v_1^2 + v_2^2 - 2r_{12}^2 v_1 v_2)/n.$$

$r_{12}$  is found to be 0.8233 for whole plots. Using this, and putting

$$v_1 = 88.613,$$

$$v_2 = 178.554,$$

$$n = 16,$$

we find

$$V_{v_1-v_2} = 2286,$$

or the standard error,

$$S_{v_1-v_2} = \sqrt{2286} = 47.8.$$

Now the sampling variance is based on 841 degrees of freedom. Its standard error is therefore

$$\sqrt{\{2.46.8^2/841\}} = 2.28,$$

and the standard error of the difference, 53.1, between the two estimates of sampling variance is

$$\sqrt{\{47.8^2 + 2.28^2\}} = 47.9.$$

Hence the difference, 53.1, barely exceeds its standard error, and is clearly insignificant.

Two interesting points emerge from this calculation. In the first place it is evident that the direct estimate is enormously more accurate than the indirect estimate, their variances being in the ratio of about 1 : 400. If, then, it is required to know the extent to which errors due to the sampling technique contribute to the total errors, it is essential that a direct estimate of the former should be made. This is only possible if at least two sampling-units are taken from each plot. In the present experiment the plots were strictly speaking only sampled in duplicate. At Rothamsted, however, it was found that the sampling-error did not differ significantly from what would have been expected if the units had been distributed wholly at random, *i.e.* if there had been about 17 sampling-units, each a single plant, instead of only 2, each of 8-9 plants.

This leads to the second interesting point - the extent to which the *accuracy* of the estimate of error depends on the number of degrees of freedom on which it is based. The *magnitude* of the sampling-error, and therefore the accuracy of the estimate of yield, is practically unaltered whether it is assumed that 2 or that 17 sampling-units are taken from each plot. But the standard error of the sampling variance is 9.01 in the first case (54 degrees of freedom), and 2.28 in the second (841 degrees of freedom). In this connection it should be noted that some of the advantages of complex sampling-units, but without the disadvantage of reducing the number of degrees of freedom, can be gained by dividing a plot into a small number of parts from each of which equal numbers of simple sampling-units are taken. The sample is the same size as before, and is still fairly representative of the plot, but there being a greater number of independent random locations, the labour of sampling is increased. If the plot is divided into  $n_1$  parts, and  $n_2$  sampling-units are taken from each part, the variance may be analysed thus:

Fraction	Degrees of freedom
Within parts	$n_1(n_2 - 1)$
Between parts	$n_1 - 1$
Total	$n_1n_2 - 1$

Hence the degrees of freedom available for estimate of error are reduced only by  $n_1 - 1$ , a number which would usually be not more than 4.

The desirability of adopting this procedure depends upon the relative importance of reducing labour and increasing the accuracy of the estimate of sampling-error: for samples of the same size the magnitude of the error should be much the same in the two cases.

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### SUMMARY.

(1) The desiderata of a sampling-method are outlined, and the particular case of sampling a large number of potato plots discussed.

(2) An analysis is made of the yields of 54 sub-plots of the Rothamsted Potato Experiment of 1928, both as estimated by a sampling-method and as determined by large-scale lifting.

(3) It is shown that most of the significant results of the experiment are obtained from the sample-yields, but that the higher standard error per plot obscures the effect of superphosphate.

(4) It is concluded that at Rothamsted 102, and at Woburn 56, plants would have to be lifted to give a sampling-error as small as 4 per cent. It would then be profitable only to sample experimental plots of 1/20th acre or more in area.

Finally it is a pleasure to record our indebtedness to Dr R. A. Fisher for much valuable advice and criticism: and to Mr H. J. G. Hines for assistance with the field work.

### REFERENCES.

- (1) EDEN, T. and FISHER, R. A. Studies in Crop Variation. IV. The Experimental Determination of the Value of Top Dressings with Cereals. *J. Agric. Sci.* (1927), **17**, 548. (*Rothamsted Memoirs*, **13**.)
- (2) CLAPHAM, A. R. The Estimation of Yield in Cereal Crops by Sampling Methods. *J. Agric. Sci.* (1929), **19**, 214-35.
- (3) FISHER, R. A. *Statistical Methods for Research Workers*. 2nd ed. Edinburgh: Oliver and Boyd, 1928.
- (4) WISHART, J. The Generalised Product Moment Distribution in Samples from a Normal Multivariate Population. *Biometrika* (1928), **20** A, 43.
- (5) ENGLENDOW, F. L. A Census of an Acre of Corn. *J. Agric. Sci.* (1926), **16**, 191.

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## A NOTE ON THE VALUE OF UNIFORMITY TRIALS FOR SUBSEQUENT EXPERIMENTS.

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It is now generally accepted that the soil of any field may be assumed to be markedly variable, and that, in consequence, treatments or varieties must be replicated in experimental trials, so that the error caused by soil differences may be estimated, and the significance of the results appreciated: variations in fertility over the area covered are always found in uniformity trials—that is to say, trials in which the whole area was treated exactly similarly, but plots in which were harvested, and the produce weighed, separately. In view of these irregularities, one possibility of increased precision appears to lie in carrying out a preliminary uniformity trial, harvesting the crop separately in the various plots to be used for a subsequent experiment, and so mapping out the fertility of the field by the plot yields when all are treated alike: these figures could be used later to correct the yields of the plots when under experiment, and so to circumvent to a certain extent the field experimentalist's bugbear—soil heterogeneity: it is conceivable that in this way a degree of precision might be attained with the actual experiment which could only be reached otherwise by a greater number of replicates than could well be managed, and that consequently the extra labour involved in preliminary uniformity trials might be justified. Naturally the possibilities depend entirely on the constancy of the plots in their relative productivity in different years, under different climatic conditions, and, usually, under other crops: this question could readily be explored were figures available giving the results of uniformity trials carried out over a series of years, on the same fields, sub-divided into the same plots, but such data are rare, and those discussed here are recognised as inadequate to give anything approaching a final solution. It was thought, however, that a note might serve to direct attention to the problem, and might stimulate others, who have access to suitable and more abundant data, to investigate them with the same end in view: it was also felt that some workers might be glad of an example of how the results of a uniformity trial

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might be used in subsequent experiments on the same plots, so that the method of working is described in some detail, at the risk of appearing obvious.

### THE METHOD OF USING THE INFORMATION.

If the yield of any plot under a preliminary uniformity trial (or its mean yield under a plurality of trials) be denoted by  $x$ , and its yield in the actual subsequent experiment by  $y$ , then some method of correcting  $y$  for  $x$  must be selected. At first sight the simplest procedure would appear to be to take the difference between the two—the efficacy of any one experimental treatment being tested by the mean value of  $y - x$  for the plots on which it was carried out: if  $x$  and  $y$  were expressed as percentages of the mean yield of all plots in their respective years, then a positive value of  $y - x$  would indicate that that particular treatment had been beneficial relative to the other treatments employed, and vice versa. Statistically, however, this method may lead to a loss, rather than a gain, in precision, for if  $V_x$  be the variance of  $x$ , then

$$V_{(y-x)} = V_y + V_x - 2r_{xy} \sqrt{V_x V_y}.$$

Consequently if there is no correlation between the plot yields in the two years, this method would add to the variance of  $y$  that of  $x$ : with  $V_x$  and  $V_y$  approximately equal, no gain at all would be made unless  $r_{xy}$  exceeded  $+0.5$ , whilst if it were negative there would be a serious loss.

A gain may, however, be effected by means of the regression function between the two variables: if  $V_{y \cdot x}$  denote the variance of  $y$  corrected for  $x$  by means of this (or, as it is sometimes stated “with  $x$  held constant”), then

$$V_{y \cdot x} = V_y (1 - r_{xy}^2) = V_y - \frac{(\text{Cov}_{xy})^2}{V_x},$$

where  $\text{Cov}_{xy}$  is the covariance between  $x$  and  $y$ , or the mean product of their deviations from their means. In this case there is a definite gain if  $x$  and  $y$  are at all closely related (positively or negatively)—that is, if the produce of an individual plot in one year is any guide to its performance in another. It will be realised that  $V_{y \cdot x}$  gives the variance of  $y$  corrected by the regression equation  $y = bx$ , where  $b$  is equated to  $\text{Cov}_{xy}/V_x$ : accordingly it must not be used to test the significance of the difference between the actual mean plot yields given by any two treatments, but that between the mean values of  $y - bx$  given by them: if  $n$  be the number of replicates, the figures compared will be  $S\left(\frac{y - bx}{n}\right)$ , or

$\frac{1}{n}(Sy - bSx)$ : since, with a random distribution of the treatments over the plots, the values of  $Sx$  will tend to be constant, it follows that the differences between these figures will be of the same order of magnitude as the differences between the means of  $y$ , and consequently any reduction effected in calculating  $V_{y \cdot x}$  from  $V_y$  will be a direct gain in precision. It can readily be seen that in all circumstances the variance of  $y - bx$  will be less than that of  $y - x$ , for

$$V_{y \cdot x} < V_{(y-x)},$$

$$\text{if } V_y(1 - r^2_{xy}) < V_y + V_x - 2r\sqrt{V_x V_y},$$

$$\text{if } (\sqrt{V_x} - r\sqrt{V_y})^2 > 0,$$

which is necessarily true, since a square cannot be negative.

#### THE DATA.

Uniformity trials were carried out between 1906 and 1911 on two fields at Aarslev (Denmark)(1), which provide (very limited) data to test the possibilities. One field ( $A_2$ ) was divided into 30 plots—6 strips of 5—which were all treated alike but harvested separately, and the crops grown were: 1907, oats; 1908, rye; 1909, barley; 1910, mangolds; 1911, barley. The other field ( $B_2$ ) was divided into 128 plots—16 strips of 8—and carried oats in 1906, barley in 1907, “seeds” in 1908, and rye in 1909: there was a remarkable oscillation in fertility across this field in one direction, the 1st, 3rd, . . . 15th strips consistently giving much higher yields than the 2nd, 4th, . . . 16th strips—in fact in the four years the odd numbered strips gave a total yield of 27,817, as compared to 23,383 for the even numbered strips. This oscillation apparently arose as a legacy of the old practice of ploughing in high ridges: the tops of the ridges exhibited greater fertility than the borders of the furrows, so that soil was worked from the former to the latter and the field levelled out: this meant that over the site of the old furrows there was a good depth of rich soil, whilst it was very shallow where the ridges had been. The strips were so arranged as to cover the site of the furrow and of the ridge alternately, with the result noted above: in order to escape this variation, the table was condensed by taking 2 strips together (so that the new strips each included the whole of one of the old “lands”) making it an 8 by 8 square. The paper referred to above gives the production for each individual plot as a percentage of the mean yield over the field in that year: the method of enquiry was to suppose that the last year for which figures are given was the actual

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experiment, and to calculate how far the knowledge gained by one, two, three, or four, preliminary uniformity trials would be effective in reducing the residual variance (that is, what remained after the variance that could legitimately be taken out of the total by suitable methods of "local control"), by which the significance of the results would be judged.

There appeared to be two printer's errors in the paper from which the figures were taken: with Field A<sub>2</sub> the yields given for the year 1908 add up to 3010 instead of 3000: reference to the Fig. 6 given there seemed to indicate that the excess lay in row 3 and eventually it was decided to reduce plots 3 *c* and 3 *f* to 96 and 84 respectively: again, with Field E<sub>2</sub> in 1908, column 10 sums to 791 instead of 786 as shown: reference to Fig. 13 indicated that the yield of plot 10 *g* should probably have been 92 instead of 97. These two slight changes in the data could, of course, have no appreciable effect on the results.

*Field A<sub>2</sub>.* The following table shows the arrangement of the plots on this field, together with their yields in the supposedly experimental year (1911): the thick lines divide the area into 5 blocks of 6 plots each, suitable for testing six different treatments. These would be assigned to the particular plots wholly at random, with the one restriction that each treatment must occur once in each block: the letters show an arrangement arrived at in this way with treatments denoted by the letters A to F—obtained by entering the letters separately on to six cards, and then shuffling them thoroughly for each block, and writing them down in the order in which the cards were found: since these are dummies they are left out in the working, and only referred to later as an example of the way in which previous information would be used.

D 103	A 101	B 95	D 104	C 109	F 115
B 98	C 102	E 97	A 111	D 109	E 104
F 94	E 106	F 96	C 95	B 112	A 94
C 90	B 111	E 100	C 96	F 99	B 86
D 95	A 105	F 101	A 90	E 98	D 84

Using the methods evolved by Dr Fisher<sup>(2)</sup>, the variance in this year was analysed in the following way:

Sum of the squares of the yields on 30 plots = 301,730.

Sum of the squares of the 5 block totals = 1,804,082.

From these the sums of the squares of the deviations were calculated as

$$\text{Total} - S(y - \bar{y})^2 = 301,730 - 30 \times 100^2 = 1730.$$

$$\text{Between blocks} = 6S(\bar{y}^1 - \bar{y})^2 = 6 \times \frac{1}{6^2} (1,804,082 - 5 \times 600^2) = 680,333,$$

giving the following analysis:

	Degrees of freedom	Sum of the squares	Variance	S.D.	Log <sub>e</sub> S.D.
Between blocks	4	680,333	170,083	13.0416	2.5683
Within blocks	25	1049,667	41,987	6.4798	1.8687
Total	29	1730,000	59,655	7.7237	—

If, then, 1911 stood by itself the variance of 1 plot due to experimental error would be 41.987, and the significance of the differences between means of 5 replicates would be judged by comparing them to

$\sqrt{\frac{2 \times 41.987}{5}}$ . It is seen that the restriction imposed by the block method of local control has been successful in reducing the variance from 59.655 to 41.987; comparing the variance between blocks with that within blocks,  $z = 0.6996$ , which, with  $n_1 = 4$ ,  $n_2 = 25$ , gives a value of  $P$  lying between 0.01 and 0.05, showing that there were real differences between the average fertility of the blocks.

Treating the other years similarly, the variances within the blocks were found to be as follows:

1910 ...	...	...	...	82.947
Mean, 1909-1910	...	...	...	29.343
„ 1908-9-10	...	...	...	37.785
„ 1907-8-9-10...	...	...	...	28.705

The sum of the products of the yields in any of these and the yields in 1911 can be found exactly similarly to the sum of the squares in any one year, and, having subtracted that between blocks from the total, and divided by 25, the following covariances were found within the blocks:

1910 × 1911	...	...	...	+ 38.020
1909-10 × 1911	...	...	...	+ 25.133
1908-9-10 × 1911	...	...	...	+ 31.053
1907-8-9-10 × 1911	...	...	...	+ 26.347

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Substituting the 1910 ( $x$ ) and 1911 ( $y$ ) figures in the formula given above, we obtain

$$V_{y \cdot x} = V_y - \frac{(\text{Cov}_{xy})^2}{V_x} = 41.987 - \frac{(38.020)^2}{82.947} = 24.560.$$

If, however, yields are to be corrected by a linear regression, one more degree of freedom is used up, and consequently we have, finally

$$V_{y \cdot x} = 24.560 \times \frac{25}{24} = 25.583.$$

Thus the variance of  $y - bx$  is some 40 per cent. less than that of  $y$ : the precision obtained by correcting for the 1910 yields can be compared to that of 1911 alone by considering the number of replicates that would be necessary to reduce the variance of the mean to the same point in each case: these are clearly in the proportion 25.583 : 41.987, that is as 1 : 1.642.

The full results obtained with this field were as follows:

Trial years ( $x$ )	$V_{y \cdot x}$	Relative precision	Gain in precision
—	41.987	1.000	—
1910	25.583	1.642	0.642
1909-10	21.311	1.969	0.327
1908-9-10	17.152	2.445	0.476
1907-8-9-10	18.547	2.262	- 0.183

There is a progressive lowering of the value of  $V_{y \cdot x}$  as one, two and three previous years are taken into consideration, until a point is reached at which the experiment is nearly  $2\frac{1}{2}$  times as exact as if no previous uniformity trials had been carried out: the inclusion of 1907 does not, however, improve on this, giving, in fact, a slightly higher value. The year 1909 is of some special interest as then the field carried the same crop (barley) as in the supposedly experimental year: taking only that one into consideration  $V_{y \cdot x}$  was found to be 35.904, so that a different crop in the preceding year was a much better basis for correction than the same crop two years before.

*Field E<sub>2</sub>.* By putting 2 strips together as mentioned above, the plots in this field were reduced to 64, and were in the form of an 8 by 8 square: with such plots an experiment might be set out in the form of a Latin square, or simply by dividing into 8 blocks of 8 plots each, and the efficacy of previous uniformity trials was tested under both of these arrangements. Taking the Latin square first, the simple addition of the squares of the 1909 yields gave the following:

64 plots	...	...	...	...	2,566,204
Totals of 8 columns	...	...	...	...	20,517,636
Totals of 8 rows	...	...	...	...	20,482,768

Then, since the mean plot yield was 200 and the mean total yield of rows and columns 1600, we have the following as the contributions to the sum of the squares:

$$\begin{aligned}\text{Total} &= 2,566,204 - 64 \times 200^2 = 6204 \\ \text{Columns} &= \frac{1}{8} (20,517,636 - 8 \times 1600^2) = 4704.5 \\ \text{Rows} &= \frac{1}{8} (20,482,768 - 8 \times 1600^2) = 346\end{aligned}$$

giving the following analysis

	Degrees of freedom	Sum of the squares	Variance	S.D.	Log <sub>e</sub> S.D.
Between columns	7	4704.5	672.071	25.9244	3.2553
Between rows	7	346.0	49.429	7.0306	1.9503
Error	49	1153.5	23.541	4.8519	1.5794
Total	63	6204.0	98.476	9.9235	—

The effect of correcting  $y$  for  $x$  by linear regression on the variance ascribable to experimental error, in the case of this arrangement on this field, was as follows:

Trial years ( $x$ )	$V_{y \cdot x}$	Relative precision	Gain in precision
—	23.541	1.000	—
1908	23.129	1.018	0.018
1907-8	23.737	0.992	- 0.026
1906-7-8	23.981	0.981	- 0.011

It is clear that in this field the plots did not tend to keep their relative yield positions from year to year, so that uniformity trials could serve no useful purpose to correct the yields under experiment: there was in fact a slight loss in precision in the last two cases, which arose by the elimination of one more degree of freedom, for if the covariance is zero,  $V_{y \cdot x}$  will obviously be  $V_y \times \frac{49}{48}$ .

In the above case a large part of the sum of squares was taken out in the columns, so that it would appear possible that previous information might have been valuable if the experiment had been planned on the block system, though that design itself would not have been so efficient: with 8 blocks of 8 plots each, however, there was again no reduction of variance by correction for previous yields:

Trial years ( $x$ )	$V_{y \cdot x}$	Relative precision	Gain in precision
—	42.571	1.000	—
1908	41.106	1.035	0.035
1907-8	42.088	1.011	- 0.024
1906-7-8	42.989	0.990	- 0.021

It will be noticed that the variances are much larger in this case, showing that the Latin square method of local control would be much more effective than that of blocks—though 2 strips (2 “columns”)

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were taken together there yet remained a large variation across that direction.

With the original 128 plots of this field it is unlikely that any experiment would have been laid down with no local control, either by strips or by blocks: if, however, treatments had been completely randomised over the whole field, so that the fertility oscillation in the columns could not have been taken out, the experiment would have lost much in precision, but a considerable proportion would have been retrieved by taking the regression on previous uniformity trials into account: for all the deviations caused by soil variations would have gone into error, and, as the relative yields of the plots in the odd numbered columns were consistently high, whilst those in the even numbered columns were low, a large part of that variance would be eliminated in calculating  $V_{v \cdot x}$  from  $V_v$ . This serves to emphasise the point that uniformity trials will be more effective in increasing precision in subsequent experiments on very irregular soils, and where local fluctuations are not adequately controlled by the design of the experiment.

### EXPERIMENTAL TRIAL.

Although preliminary uniformity trials would be futile on Field E<sub>2</sub> (that is, if reasonable experimental methods were adopted), they would give valuable information on Field A<sub>2</sub>, as it appears that there were variations between individual plots (as distinct from those between blocks or strips) of a more or less constant nature—though as to whether this was a matter of plant food, soil texture, drainage, etc., we have no information. The greatest reduction of the variance due to error was given by correction from the yields in the previous three years, so that it was thought desirable to see exactly how the inclusion of this regression would affect the analysis: the random distribution of six dummy treatments is shown on p. 66. The regression of  $y$  on  $x$  is given by  $\frac{\text{Cov}_{xy}}{V_x}$ , which in this case takes the value  $\frac{38.682}{41.84645}$  or 0.92438. The various treatments gave:

	Mean plot yield ( $y$ )	Mean yield of same plots in 1908–9–10 ( $x$ )	( $y - bx$ )
A	100.2	98.2	9.4
B	100.4	98.8	9.1
C	98.4	100.47	5.5
D	99.0	100.2	6.4
E	101.0	103.8	5.0
F	101.0	98.53	9.9

For actual yields the greatest difference is between E or F and C, and amounts to 2.6: with corrected yields it is seen that E was favoured

by the soil allotted to it, and that it actually evoked the least response, differing from F by 4.9.

Neglecting, at first, the regression, the analysis of variance (of  $y$ ) is as follows:

	Degrees of freedom	Sum of the squares	Variance	S.D.	Log <sub>e</sub> S.D.
Between blocks	4	680.333	170.083	13.0416	2.5683
Between treatments	5	28.800	5.760	2.4000	0.8755
Error	20	1020.867	51.0435	7.1445	1.9663
Total	29	1730.000	59.655	7.7237	—

The random distribution of treatments has been peculiar in giving such small differences: comparing the treatment variance with that due to error,  $z = -1.0908$ , which, with  $n_1 = 20$  and  $n_2 = 5$ , gives a value of  $P$  lying between 0.01 and 0.05, showing that such evenness would be given by chance less than 5 times in 100: since practically nothing is taken out by treatment, and 5 degrees of freedom are sacrificed to it, the variance due to error is raised.

The variance of  $(y - bx)$  may be determined directly by preparing a fresh table, giving instead of the experimental yield of each plot the value of  $(y - bx)$ , and by obtaining and analysing the sum of the squares from these new figures. Alternatively it may be derived from tables already calculated for the analysis of the variance of  $y$ , and of  $x$ , and of the covariance of  $xy$ : each of these will contain four rows—one showing the variance (or covariance) between blocks, one between treatments (these of course will always be purely hypothetical in the case of  $x$ ), one for experimental error, and one for the total. For the compound observation  $(y - bx)$  we have

$$S(y - bx)^2 = S(y^2) - 2bS(xy) + b^2S(x^2),$$

so that the new table can be constructed by applying this formula to the other three tables, row for row all through. Since  $(y - bx)$  contains one statistic ( $b$ ) already obtained from the data, the total number of degrees of freedom will be reduced from 29 to 28, and, as  $b$  has been calculated from the figures for error, this one degree of freedom will be taken from that row. In this way the following analysis of the variance of  $(y - bx)$  was obtained:

	Degrees of freedom	Sum of the squares	Variance	S.D.	Log <sub>e</sub> S.D.
Between blocks	4	531.133	132.783	11.5230	2.4444
Between treatments	5	115.854	23.171	4.8136	1.5715
Error	19	305.727	16.0909	4.0113	1.3890
Total	28	952.714	34.0255	5.8331	—

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In this case our random distribution of treatments shows no peculiarities, the treatment variance agreeing closely with that due to error.

The comparative efficiency in the two cases can be seen from the following:

					Without regression	With regression
Variance of 1 plot	...	...	...	...	51.0435	16.0909
Variance diff. of means of 5 plots					20.4174	6.4364
S.E.	...	...	...	...	4.5186	2.5370

A table of  $t$  shows that  $P = 0.01$  when  $t = 2.845$  with 20 degrees of freedom, and when  $t = 2.861$  with 19 degrees of freedom, so that a difference of 12.86 would be needed for this standard of significance where the regression was not introduced, but only one of 7.26 when that part of the variance accounted for by it was eliminated. As shown by the values of  $z$  in the two cases, the former is not approached by any of the differences between the treatment means, neither is the latter by any of the differences between the mean values of  $(y - bx)$ : this was to be expected since the treatments were dummies, but had real treatments been carried out it will be seen that the use of the regression would have materially increased the exactitude of the comparison—in fact under the latter conditions 6 replicates would have provided as precise information as 19 would have done if the experimental year gave our only measure of the productivity of the plots. This present insertion of hypothetical treatments into the data shows a greater increase in precision than indicated by the value of  $V_{y \cdot x}$  on p. 68, where the relative accuracy in the case of these three preliminary trials was shown to be as 1 : 2.445: the discrepancy arises in the chance distribution of the treatments over the plots, and the value given there for  $V_{y \cdot x}$  is the more definite, for that shows what would be the average figure, if a large number of trials with dummy treatments, such as the present, were carried out.

In this instance the gain is very considerable, and it is possible that under certain circumstances (*e.g.* with a restricted area, or where little assistance was available at any one time) it might repay the labour of three years uniformity trials, even though it would increase the work four-fold and the precision but little more than three-fold: such a result must not however be expected in all cases, for with Field  $E_2$ , as shown above, the yields under previous trials would be quite ineffective as a basis for correction. The final decision of this question of the value of uniformity trials in this direction must await the analyses of a number of series of uniformity trials carried out on other fields, and it will vary

in each particular case according to the degree of soil heterogeneity of the experimental area, and the adequacy of the methods of local control that are adopted: it is thought however that the present results, meagre though they are, suffice to indicate that in some cases appreciable advances might possibly be made along this line.

#### SUMMARY.

The question attacked is whether soil variations are sufficiently constant from year to year to give useful corrections to the yields of experimental plots from their yields under previous uniformity trials, and the data investigated were the published results of uniformity trials carried out on two fields at Aarslev (Denmark) between 1906 and 1911. In one case the plots did tend to keep constant in their relative yields, and the precision of an experiment would be increased by nearly 150 per cent. if the regression on the mean yield in the three previous years were used: with the other field, however, the plots showed no constancy in yield (when the variation due to strips was taken out as in modern experimental methods), and consequently previous uniformity trials could give no assistance.

The work described here was done whilst I was enjoying the hospitality of the Rothamsted Laboratory: it gives me much pleasure to acknowledge my great indebtedness to Dr R. A. Fisher, F.R.S., who suggested the problem, and guided my unsteady steps throughout.

#### REFERENCES.

- (1) HANSON, N. A. "Provedyrkning paa Forsogsstationen ved Aarslev." *Tids. for Landbrugets Planteavl* (1914), **21**, 553.
- (2) FISHER, R. A. *Statistical Methods for Research Workers* (1928). Edinburgh, 2nd edition.

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THE GENERAL SAMPLING DISTRIBUTION OF THE  
MULTIPLE CORRELATION COEFFICIENT.

BY

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*The General Sampling Distribution of the Multiple Correlation Coefficient.*

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1. *Introductory.*

Of the problems of the exact distribution of statistics in common use that of the multiple correlation coefficient is the last to have resisted solution. It will be seen that the solution introduces an extensive group of distributions, occurring naturally in the most diverse types of statistical investigation, and which in their mere mathematical structure supply an extension in a new direction of the entire system of distributions previously obtained, which in their generality underlie the analysis of variance. The individual distributions of this system were in each case obtained by the exact investigation of a particular problem. It was realised only gradually that many of these distributions, disguised by the different notations appropriate to different problems, were in reality equivalent, and could be made available in practice by a single system of tables. The remaining cases, with the notable exception of the correlation coefficient, then fall into place as particular limiting forms of a single general distribution. As the practical utility of these earlier solutions depends greatly on a recognition of their place in a single system, a very brief account of their mutual relationship may be given.

The only statistic derived from samples of a continuous variate, of which the distribution was known before the present century, appears to be the arithmetic mean of a sample drawn from the normal distribution. In addition, however, two distributions which may be regarded as distributions of statistics had also been found, namely, Bernoulli's binominal distribution, and Poisson's series. Both of these distributions possess the property that the aggregate of the values of a sample is itself distributed in a distribution of the same type. In all three classical cases, therefore, the distribution of the statistic derived from a finite sample was known only by a mathematical simplification of this special type. In all other cases, approximations of unknown accuracy based on the use of the standard error and the assumption of normal distributions had perforce to be used.

In 1908 "Student"\* attacked the problem of the distribution of the mean of a normal sample measured, as in practice it must usually be, in terms of the standard error as estimated from the same sample. He was thus incidentally led to the equally fundamental distribution of the variance of a normal sample. This latter, to which the general distribution of the analysis of variance degenerates when  $n_2$  tends to infinity, is in reality equivalent to the distribution found by Pearson† in 1900 for the  $\chi^2$  measure of discrepancy developed for testing goodness of fit. From this "Student" was able to derive the exact distribution (the distribution of  $t$ ) of the mean of a unique sample, which as subsequently appeared falls into the same system with  $n_1 = 1$ . The two principal limiting forms of the general distribution were thus known in 1908, and were available for practical application by means of Elderton's‡ and "Student's"\* first tables.

In 1915 the distribution of the coefficient of correlation was obtained§ by a use of Euclidean hyper-space similar to that employed below. The same method served at the same time to put "Student's" results upon a rigorous basis. The distribution of the correlation coefficient stands outside the analysis of variance system, but, as will be seen in the present paper, it is brought into coherent connection with it by the distribution of the coefficient of multiple correlation. When, however, the corresponding distribution of the intraclass correlation was obtained||, the distribution found was of a new and different type, which, as subsequently appeared, was the general distribution of the analysis of variance, in which the variance is analysed into two parts representing that within and that between the classes or "fraternities" of which the data are composed. This was the first instance of the general distribution which from the notation there used is distinguished as the distribution of  $z$ .

The recognition of the fundamental importance of the two parameters,  $n_1$  and  $n_2$ , which specify the numbers of degrees of freedom in the two estimates of variance to be compared, and the recognition of the distribution of  $\chi^2$  as equivalent to that of an estimate of variance led, in 1922 and the following two years,¶ to the demonstration that it is always the number of degrees of freedom

\* 'Biometrika,' vol. 6, p. 1 (1908).

† 'Phil. Mag.,' vol. 50, p. 157 (1900).

‡ 'Biometrika,' vol. 1, p. 155 (1902).

§ Fisher, 'Biometrika,' vol. 10, p. 507 (1915).

|| 'Metron,' vol. 1, No. 4, p. 1 (1921).

¶ 'J. R. Stat. Soc.,' vol. 85, p. 87 (1922); 'Economica,' vol. 3, p. 139 (1923); 'J. R. Stat. Soc.,' vol. 87, p. 442 (1924).

which is to be used in applying the test of goodness of fit. The further proof that the test is only valid when the methods of estimation employed have been *efficient*, binds the theory of goodness of fit closely to that of estimation in the development of which the exact distribution of statistics play an essential part.\*

Meanwhile,† a solution of the exact distribution of  $\chi^2$  when applied to test the goodness of fit of regression formulæ had shown that a modification was required in this case, which, in fact, involved dropping the approximate assumption that  $n_2$  was infinite, and reduced the general distribution to the same form as that already found in the study of intraclass correlation. At the same time, the distribution of the correlation ratio,  $\eta$ , derived from uncorrelated material, was shown to belong to the same class with  $n_1$ , equal to one less than the number of arrays; and the distribution of regression coefficients, whether total or partial, and whether employed in a linear or a non-linear formula, were shown to conform to "Student's" distribution. The solution of the distribution of the correlation ratio  $\eta$  really included also that of the multiple correlation coefficient for samples drawn from uncorrelated material, the distribution of which was given in its appropriate notation in 1924.‡

In the same year§ it was found possible to use the representation in hyperspace to demonstrate that the distribution of the partial correlation coefficients is exactly the same as that primarily found for the total correlation, provided that unity is deducted from the sample number for each variate eliminated.

Each distinct type of distribution found has thus occurred repeatedly in different investigations; whereas, however, nearly all cases are reducible to a common type capable of exact treatment by the same simple arithmetical procedure,|| and requiring the same fundamental table, the distribution of the (intraclass) correlation coefficient, total or partial, stood aside from the main system, and was capable of only an approximate treatment by using the distribution of  $z$ .

The distribution of the multiple correlation coefficient, apart from the practical necessity of applying to observed results sufficiently exact tests of significance, is thus of great theoretical interest owing to the close connection which must exist between it and the simple correlation coefficient, on the one hand, and, on the other, to the form already obtained from uncorrelated material.

\* 'Phil. Trans.,' A, vol. 222, p. 309 (1922).

† 'J. R. Stat. Soc.,' vol. 85, p. 597 (1922).

‡ 'Phil. Trans.,' B, vol. 213, p. 89 (1924).

§ 'Metron.,' vol. 3, p. 329 (1924).

|| 'Statistical Methods for Research Workers,' 2nd ed., Oliver & Boyd, Edinburgh, 1928.

The latter solution involves, besides the variate and frequency, the two parameters  $n_1$  and  $n_2$ , and is therefore a functional relation between four variables. The new solution necessarily involves also the multiple correlation in the population sampled, making a fifth variable; complete tabulation of the results would thus require a table of fourfold entry; even confining attention to specified points of special importance, such as the 5 per cent. and 1 per cent. points, a procedure that has made tabulation practicable for the distribution of  $z$ , we should still have tables of triple entry. The problem of adequate tabulation is certainly not insurmountable, but to ascertain the proper method to adopt in its presentation will require further study of the nature of the function. The table of the 5 per cent. points of the distribution of  $B$  (Section 5) should in the author's opinion provide sufficient guidance for the greater number of practical applications.

## 2. *Method of Solution.*

The primary problem of the sampling distribution of the correlation coefficient between two variates,  $x$  and  $y$ , was originally solved by interpreting the  $n$  individual values of either variate appearing in the sample as the co-ordinates of a point in Euclidean space of  $n$  dimensions. It then easily appeared that the correlation coefficient between the variates was the cosine of the angle between the two *radii vectores* drawn from the origin to points, the co-ordinates of which represented the deviations from the mean of the sample of the two variates concerned.

The frequency with which  $r$ , the observed correlation coefficient, falls in any infinitesimal range  $dr$  may be usefully thought of as the product of two factors, one being the generalised volume in which the second sample point may lie so that the correlation may fall within the assigned range, this value being independent of the correlational properties of the population sampled, while the second is a factor by which the frequency density in any element of volume is modified by the correlation between  $x$  and  $y$  in the population. With zero correlation in the population, the frequency density at any point depends only on its distance from the origin, and since for any given distance the point is free to move over a sphere in  $(n - 1)$  dimensions, one dimension having been eliminated by using the sample mean as origin, it is easy to see that for this case the frequency distribution of  $r$  is given by

$$df = \frac{[\frac{1}{2}(n-3)]!}{[\frac{1}{2}(n-4)]! \sqrt{\pi}} (1-r^2)^{\frac{1}{2}(n-4)} dr.$$

The general solution of the primary problem\*

$$df = \frac{n-2}{\pi} (1-\rho^2)^{\frac{1}{2}(n-1)} (1-r^2)^{\frac{1}{2}(n-4)} \int_0^{\infty} \frac{dz}{(\cosh z - \rho r)^{n-1}} \cdot dr,$$

may be written with advantage

$$df = \frac{[\frac{1}{2}(n-3)]!}{[\frac{1}{2}(n-4)]! \sqrt{\pi}} (1-r^2)^{\frac{1}{2}(n-4)} dr \\ \times \frac{[\frac{1}{2}(n-2)]!}{[\frac{1}{2}(n-3)]! \sqrt{\pi}} (1-\rho^2)^{\frac{1}{2}(n-1)} \int_{-\infty}^{\infty} \frac{dz}{(\cosh z - \rho r)^{n-1}}.$$

The second factor then represents the effect upon the frequency density, in the region represented by  $dr$ , of a correlation  $\rho$  in the sampled population: the numerical part of this factor is merely such as to reduce it to unity when  $\rho = 0$ .

With multiple correlations we are concerned with the correlations between a dependent variate  $y$ , and a number of independent variates,  $x_1, x_2, \dots, x_n$ , and, moreover, with the correlations of the latter among themselves. It was not at first obvious that the sampling distribution did not involve this whole matrix of correlations, in which case, even if it could be determined, it would be of no practical use. The argument, by which it can be seen to depend from only a single parameter of the population, is therefore of special interest, as by its general character it is applicable to a number of statistical problems, and leads in this case directly to the solution.

The multiple correlation of  $y$  with  $x_1, x_2, \dots, x_n$  is the correlation between  $y$  and that linear function of  $x_1, x_2, \dots, x_n$  with which its correlation is highest. If, therefore, for the dependent variates,  $x$ , we substitute an equal number of new variates,  $\xi$ , defined as linear functions of the  $n_1$  variates,  $x$ , then the multiple correlation in the population, and in every sample from it, will remain unchanged. In particular we may choose as  $\xi_1$ , that linear function the correlation of which with  $y$  in the population sampled is highest, and for the remaining variates,  $\xi$ , we can choose linear functions of  $x$ , uncorrelated with  $\xi_1$ , or with each other. In choosing the last of these we have no more than  $n_1 - 1$  conditions to be satisfied by the ratios of  $n_1$  coefficients. If this is done it is easy to see, or to demonstrate, that all of the variates  $\xi$ , except  $\xi_1$ , have zero correlation with  $y$ . Using the variates  $\xi$  the sampling distribution of the multiple correlation  $R$  can only depend on the correlation in the population sampled between  $\xi_1$  and  $y$ , namely, on the multiple correlation in the population sampled, which we may designate by  $\rho$ .

\* Fisher, 'Biometrika,' vol. 10, p. 507 (1915).

The geometrical interpretation of the multiple correlation coefficient  $R$  is that it is the cosine of the angle between the *radius vector* of the dependent variate  $y$  and the planar region including the *radii vectores* of the  $n_1$  independent variates; its distribution when  $\rho = 0$ , which depends only on the geometrical elements of volume, has been thus shown\* to be

$$df = \frac{[\frac{1}{2}(n_1 + n_2 - 2)]!}{[\frac{1}{2}(n_1 - 2)]! [\frac{1}{2}(n_2 - 2)]!} (R^2)^{\frac{1}{2}(n_1 - 2)} (1 - R^2)^{\frac{1}{2}(n_2 - 2)} d(R^2),$$

where  $n$ , the sample number, is replaced by  $n_1 + n_2 + 1$ ; but in what way this distribution is modified when  $\rho$  is not zero has been hitherto entirely unknown.

It is evident, however, that since we have reduced the problem of the multiple correlation coefficient to one involving only a single correlation, the frequency density of any configuration will be affected merely by a factor

$$\frac{[\frac{1}{2}(n - 2)]!}{[\frac{1}{2}(n - 3)]! \sqrt{\pi}} (1 - \rho^2)^{\frac{1}{2}(n-1)} \int_{-\infty}^{\infty} \frac{dz}{(\cosh z - \rho r)^{n-1}},$$

in which  $r$  is the correlation in the sample between  $y$  and  $\xi_1$ ; this factor will, however, vary, because  $r$  varies in the different configurations which give rise to the same value of  $R$ . Consider now a third variate,  $Y$ , representing the linear function of the independent variates which in the sample is most closely correlated with  $y$ , or, in other words, the prediction formula for  $y$ . Its correlation with  $\xi_1$  we may represent by  $\cos \psi$ , and since the partial correlation of  $y$  with  $\xi_1$  (or any other linear function of the independent variates) when  $Y$  is eliminated, must be zero, it is evident that

$$r = R \cos \psi.$$

For a given value of  $\psi$ , therefore, the density factor is constant in the different configurations which give the same value of  $R$ , but, in the absence of correlation, the frequency with which  $\psi$  falls in the range  $d\psi$  is evidently

$$\frac{[\frac{1}{2}(n_1 - 2)]!}{[\frac{1}{2}(n_1 - 3)]! \sqrt{\pi}} \sin^{n_1-2} \psi d\psi;$$

hence integrating over all values of  $\psi$ , the density factor becomes

$$\frac{(1 - \rho^2)^{\frac{1}{2}(n_1 + n_2)}}{\pi} \cdot \frac{[\frac{1}{2}(n_1 + n_2 - 1)]!}{[\frac{1}{2}(n_1 + n_2 - 2)]!} \\ \times \frac{[\frac{1}{2}(n_1 - 2)]!}{[\frac{1}{2}(n_1 - 3)]!} \int_0^\pi d\psi \int_{-\infty}^{\infty} \frac{\sin^{n_1-2} \psi \cdot dz}{(\cosh z - \rho R \cos \psi)^{n_1 + n_2}},$$

\* Fisher, 'Phil. Trans.,' B, vol. 213, p. 89 (1924).

and the complete expression for the distribution of  $R$  is

$$df = \frac{[\frac{1}{2}(n_1 + n_2 - 1)]!}{[\frac{1}{2}(n_2 - 2)]! [\frac{1}{2}(n_1 - 3)]!} \cdot \frac{(1 - \rho^2)^{\frac{1}{2}(n_1 + n_2)}}{\pi} \\ \times (R^2)^{\frac{1}{2}(n_1 - 2)} (1 - R^2)^{\frac{1}{2}(n_1 - 2)} d(R^2) \int_0^\pi d\psi \int_{-\infty}^\infty \frac{\sin^{n_1 - 2} \psi \cdot dz}{(\cosh z - \rho R \cos \psi)^{n_1 + n_2}}.$$

### 3. The Hypergeometric Form.

Apart from the factor,

$$(1 - \rho^2)^{\frac{1}{2}(n_1 + n_2)},$$

the density factor may be reduced to a hypergeometric function. For in the expression,

$$\frac{1}{\pi} \cdot \frac{[\frac{1}{2}(n_1 + n_2 - 1)]!}{[\frac{1}{2}(n_1 + n_2 - 2)]!} \cdot \frac{[\frac{1}{2}(n_1 - 2)]!}{[\frac{1}{2}(n_1 - 3)]!} \int_0^\pi d\psi \int_{-\infty}^\infty \frac{\sin^{n_1 - 2} \psi \cdot dz}{(\cosh z - \rho R \cos \psi)^{n_1 + n_2}},$$

the integrand may be expanded in the uniformly convergent series

$$\sum_{t=0}^\infty \frac{(n_1 + n_2 + 2t - 1)!}{(n_1 + n_2 - 1)! (2t)!} \cdot \frac{\cos^{2t} \psi \sin^{n_1 - 2} \psi}{\cosh^{n_1 + n_2 + 2t} z} (\rho^2 R^2)^t,$$

in which the odd powers of  $\cos \psi$ , which evidently disappear on integration, have been omitted. Remembering now that

$$\int_0^\pi \cos^{2t} \psi \sin^{n_1 - 2} \psi d\psi = \frac{[\frac{1}{2}(2t - 1)]! [\frac{1}{2}(n_1 - 3)]!}{[\frac{1}{2}(n_1 + 2t - 2)]!},$$

and

$$\int_{-\infty}^\infty \frac{dz}{\cosh^{n_1 + n_2 + 2t} z} = \frac{[\frac{1}{2}(n_1 + n_2 + 2t - 2)]! \sqrt{\pi}}{[\frac{1}{2}(n_1 + n_2 + 2t - 1)]!},$$

we have a power series for the integral, which may be written

$$\frac{[\frac{1}{2}(n_1 - 2)]!}{[\frac{1}{2}(n_1 + n_2 - 2)]!^2} \sum_{t=0}^\infty \frac{[\frac{1}{2}(n_1 + n_2 + 2t - 2)]!^2}{t! [\frac{1}{2}(n_1 + 2t - 2)]!} (\rho^2 R^2)^t,$$

or

$$F\left[\frac{1}{2}(n_1 + n_2), \frac{1}{2}(n_1 + n_2), \frac{1}{2}n_1, \rho^2 R^2\right],$$

so that the distribution of  $R$  obtained in section 2 takes the form

$$df = \frac{[\frac{1}{2}(n_1 + n_2 - 2)]!}{[\frac{1}{2}n_1! (n_1 - 2)!] [\frac{1}{2}(n_2 - 2)]!} (1 - \rho^2)^{\frac{1}{2}(n_1 + n_2)} F\left[\frac{1}{2}(n_1 + n_2), \frac{1}{2}(n_1 + n_2), \frac{1}{2}n_1, \rho^2 R^2\right] \\ \times (R^2)^{\frac{1}{2}(n_1 - 2)} (1 - R^2)^{\frac{1}{2}(n_1 - 2)} d(R^2). \quad (A)$$

## 4. Elementary Cases.

4.1. *When  $n_2$  is even.*—When  $n_2$  is even the identity,

$$F\left[\frac{1}{2}(n_1 + n_2), \frac{1}{2}(n_1 + n_2), \frac{1}{2}n_1, \rho^2 R^2\right] \\ = (1 - \rho^2 R^2)^{-\frac{1}{2}(n_1 + 2n_2)} = F\left(-\frac{1}{2}n_2, -\frac{1}{2}n_2, \frac{1}{2}n_1, \rho^2 R^2\right),$$

gives a terminating series.

Thus, when  $n_2 = 2$ , we have the series of distributions

$$df = (1 - \rho^2)^{\frac{1}{2}(n_1 + 2)} (1 - \rho^2 R^2)^{-\frac{1}{2}(n_1 + 4)} (n_1 + 2\rho^2 R^2) R^{n_1 - 1} dR,$$

having the special forms

$$(2.2) \quad df = (1 - \rho^2)^2 (2 + 2\rho^2 R^2) (1 - \rho^2 R^2)^3 \cdot R dR,$$

$$(3.2) \quad df = (1 - \rho^2)^{5/2} (3 + 2\rho^2 R^2) / (1 - \rho^2 R^2)^{7/2} \cdot R^2 dR,$$

$$(4.2) \quad df = (1 - \rho^2)^3 (4 + 2\rho^2 R^2) / (1 - \rho^2 R^2)^4 \cdot R^3 dR$$

when  $n_1$  is 2, 3 and 4.

When  $n_2 = 4$ , we have a somewhat less simple series of distributions

$$df = \frac{2(1 - \rho^2)^{\frac{1}{2}(n_1 + 4)}}{(1 - \rho^2 R^2)^{\frac{1}{2}(n_1 + 8)}} \left\{ \frac{n_1(n_1 + 2)}{2 \cdot 4} + 2 \frac{n_1 + 2}{2} \rho^2 R^2 + \rho^4 R^4 \right\} R^{n_1 - 2} (1 - R^2) d(R^2),$$

and when  $n_2 = 6$ , a series which may be written

$$df = \frac{3(1 - \rho^2)^{\frac{1}{2}(n_1 + 6)}}{(1 - \rho^2 R^2)^{\frac{1}{2}(n_1 + 12)}} \left\{ \frac{n_1(n_1 + 2)(n_1 + 4)}{2 \cdot 4 \cdot 6} + 3 \frac{(n_1 + 2)(n_1 + 4)}{2 \cdot 4} \rho^2 R^2 \right. \\ \left. + 3 \frac{n_1 + 4}{2} \rho^4 R^4 + \rho^6 R^6 \right\} R^{n_1 - 2} (1 - R^2)^2 d(R^2),$$

an expression in which the general method of formation of the terms is readily seen.

4.2. *When  $n_1$  and  $n_2$  are both odd.*—A second group of cases in which the frequency element is expressible in finite terms in elementary functions occurs when both  $n_1$  and  $n_2$  are odd. If, for example, we put  $n_1 = 3$  in the expression

$$\int_0^\pi d\psi \int_{-\infty}^\infty \frac{\sin^{n_1 - 2} \psi \cdot dz}{(\cosh z - \rho R \cos \psi)^{n_1 + n_2}},$$

and integrate with respect to  $\cos \psi$ , we obtain

$$\int_{-\infty}^\infty \frac{dz}{(n_2 + 2) \rho R} \{(\cosh z - \rho R)^{-(n_1 + 2)} - (\cosh z + \rho R)^{-(n_1 + 2)}\},$$

a form of integral which, as was shown in the case of the simple correlation coefficient\*, is expressible in finite terms by the aid of the circular functions.

\* Fisher, 'Biometrika,' vol. 10, p. 507 (1915).

For

$$\int_{-\infty}^{\infty} \frac{dz}{\cosh z - \rho R} = \frac{2\theta}{\sin \theta},$$

where  $\cos \theta = -\rho R$ , and  $\theta$  does not exceed the bounds 0 to  $\pi$ ; hence

$$\int_{-\infty}^{\infty} \frac{dz}{(\cosh z - \rho R)^{n_2+2}} = \frac{2}{(n_2+1)!} \left( \frac{d}{d \cos \theta} \right)^{n_2+1} \frac{\theta}{\sin \theta},$$

and therefore, if  $n_2$  is odd,

$$\begin{aligned} \frac{1}{(n_2+2)\rho R} \int_{-\infty}^{\infty} \{(\cosh z - \rho R)^{-(n_2+2)} - (\cosh z + \rho R)^{-(n_2+2)}\} dz \\ = \frac{4}{(n_2+2)! \rho R} \left( \frac{d}{d(\rho R)} \right)^{n_2+1} \frac{\sin^{-1} \rho R}{\sqrt{1 - \rho^2 R^2}}. \end{aligned}$$

Hence for the determination of the simpler distributions of this series we require

$$\begin{aligned} \left( \frac{d}{\cos \phi d\phi} \right)^2 \frac{\phi}{\cos \phi} &= \frac{1}{\cos^3 \phi} (\phi + 3 \tan \phi + 3\phi \tan^2 \phi) \\ \left( \frac{d}{\cos \phi d\phi} \right)^4 \frac{\phi}{\cos \phi} &= \frac{1}{\cos^5 \phi} (9\phi + 55t + 90\phi t^2 + 105t^3 + 105\phi t^4) \\ \left( \frac{d}{\cos \phi d\phi} \right)^6 \frac{\phi}{\cos \phi} &= \frac{9}{\cos^7 \phi} (25\phi + 231t + 525\phi t^2 + 1190t^3 \\ &\quad + 1575\phi t^4 + 1155t^5 + 1155\phi t^6), \end{aligned}$$

which lead directly to the distributions,

$$(3.1) \quad df = \frac{1}{\pi} (1 - \rho^2)^2 (1 - R^2)^{-\frac{1}{2}} (1 - \rho^2 R^2)^{-2} \{3 + \alpha (1 + 2\rho^2 R^2)\} R^2 dR,$$

in which  $\alpha$  stands for

$$\frac{\sin^{-1}(\rho R)}{\rho R \sqrt{1 - \rho^2 R^2}} = 1 + \frac{2}{3} \rho^2 R^2 + \frac{2 \cdot 4}{3 \cdot 5} \rho^4 R^4 + \dots \text{ad inf.}$$

$$(3.3) \quad df = \frac{1}{4\pi} (1 - \rho^2)^3 (1 - R^2)^{\frac{1}{2}} (1 - \rho^2 R^2)^{-4} R^2 dR \\ \times \{5(11 + 10\rho^2 R^2) + 3\alpha(3 + 24\rho^2 R^2 + 8\rho^4 R^4)\},$$

$$(3.5) \quad df = \frac{1}{8\pi} (1 - \rho^2)^4 (1 - R^2)^{3/2} (1 - \rho^2 R^2)^{-6} R^2 dR \\ \times \{7(33 + 104\rho^2 R^2 + 28\rho^4 R^4) + 5\alpha(5 + 90\rho^2 R^2 + 120\rho^4 R^4 + 16\rho^6 R^6)\}.$$

A similar process of integration is available for other odd values of  $n_1$ ; for  $n_1 = 5$  we have the distributions

$$(5.1) \quad df = \frac{1}{4\pi\rho^2} (1 - \rho^2)^3 (1 - R^2)^{-\frac{1}{2}} (1 - \rho^2 R^2)^{-3} R^2 dR \\ \times \{1 + 14\rho^2 R^2 + \alpha(-1 + 8\rho^2 R^2 + 8\rho^4 R^4)\}.$$

$$(5.3) \quad df = \frac{3}{8\pi\rho^2} (1 - \rho^2)^4 (1 - R^2)^{\frac{1}{2}} (1 - \rho^2 R^2)^{-5} R^2 dR \\ \times \{1 + 68\rho^2 R^2 + 36\rho^4 R^4 + \alpha(-1 + 18\rho^2 R^2 + 72\rho^4 R^4 + 16\rho^6 R^6)\}.$$

$$df = \frac{1}{64\pi\rho^2} (1 - \rho^2)^5 (1 - R^2)^{3/2} (1 - \rho^2 R^2)^{-7} R^2 dR \\ (5.5) \quad \times \{25 + 4678\rho^2 R^2 + 8664\rho^4 R^4 + 1648\rho^6 R^6 \\ + \alpha(-25 + 800\rho^2 R^2 + 7200\rho^4 R^4 + 6400\rho^6 R^6 + 640\rho^8 R^8)\}.$$

The polynomial coefficient of  $\alpha$  in the hypergeometric function is itself easily expressed in terms of a function of this sort, in the forms

$$\frac{3 \cdot 5 \dots (n_1 - 2) \cdot 3^2 \cdot 5^2 \dots n_2^2}{2^3 \cdot 4^3 \dots (n_1 + n_2 - 2)^2} (-\rho^2 R^2)^{-\frac{1}{2}(n_1 - 3)} F\left[-\frac{1}{2}(n_1 + n_2 - 2), \frac{1}{2}(n_1 + n_2 - 2), \right. \\ \left. \frac{1}{2}(2 - n_1), \rho^2 R^2\right],$$

or

$$\frac{n_1 - 2}{2 \cdot 4 \dots (n_1 + n_2 - 2)} (\rho^2 R^2)^{\frac{1}{2}(n_1 + 1)} F\left[-\frac{n_2}{2}, -\frac{n_1 + n_2 - 2}{2}, 1, \frac{1}{\rho^2 R^2}\right];$$

from this the remainder may in any particular case be found fairly easily by equating coefficients in the initial terms of the expansion of

$$F\left(\frac{1}{2}(n_1 + n_2), \frac{1}{2}(n_1 + n_2), \frac{1}{2}n_1, \rho^2 R^2\right).$$

### 5. The Problem of Large Samples.

Some confusion has been caused by the fact that, while for any finite value of  $\rho$ , however small, the distribution of  $R$  will be normal for a sufficiently large sample, yet when  $\rho = 0$  the distribution is far from normal. The approximate distribution appropriate to the theory of large samples, for different values of  $\rho\sqrt{n_2}$ , may be found as follows.

If we write  $n_2\rho^2 = \beta^2$ ,  $n_2R^2 = B^2$ , and allow  $n_2$  to increase indefinitely, the limiting form taken by the general distribution is

$$df = \frac{(\frac{1}{2}B^2)^{\frac{1}{2}(n_1 - 2)}}{[\frac{1}{2}(n_1 - 2)]!} e^{-\frac{1}{2}B^2 - \frac{1}{2}\beta^2} \left\{ 1 + \frac{1}{n_1} \frac{\beta^2 B^2}{2} + \frac{1}{n_1(n_1 + 2)} \frac{\beta^2 B^2}{2 \cdot 4} + \dots \right\} d(\frac{1}{2}B^2), (B)$$

which may be written in terms of a Bessel function as

$$(B/i\beta)^{\frac{1}{2}(n_1 - 2)} e^{-\frac{1}{2}(B^2 + \beta^2)} \cdot J_{\frac{1}{2}(n_1 - 2)}(i\beta B) \cdot d(\frac{1}{2}B^2).$$

When  $n_1$  is odd, these may be reduced to elementary functions; thus for  $n_1 = 3$ , we have

$$df = \frac{1}{\sqrt{2\pi}} \frac{B}{\beta} \{e^{-\frac{1}{2}(B-\beta)^2} - e^{-\frac{1}{2}(B+\beta)^2}\} \cdot d\beta,$$

an interesting distribution which connects the extreme forms found by making  $\beta$  zero for uncorrelated populations, and large for populations with a significant though still small correlation. When  $\beta = 0$ , we have

$$df = (2/\pi)^{\frac{1}{2}} B^2 \exp(-\frac{1}{2}B^2) d\beta,$$

the distribution of  $x$  for 3 degrees of freedom, while when  $\beta$  is large,  $B$  is distributed normally about  $\beta$ , in the form

$$df = (2\pi)^{-\frac{1}{2}} \exp\{-\frac{1}{2}(B-\beta)^2\} d\beta,$$

and therefore  $R$  is distributed normally about  $\rho$ , with variance which may be equated to  $1/n_2$ .

When  $n_1 = 5$ , the system of distributions is

$$df = \frac{1}{\sqrt{2\pi}} \frac{B^2}{\beta^2} \left\{ \left(1 - \frac{1}{\beta B}\right) \exp\{-\frac{1}{2}(B-\beta)^2\} + \left(1 + \frac{1}{\beta B}\right) \exp\{-\frac{1}{2}(B+\beta)^2\} \right\} d\beta,$$

and when  $n_1 = 7$

$$df = \frac{1}{\sqrt{2\pi}} \frac{B^3}{\beta^3} \left\{ \left(1 - \frac{3}{\beta B} + \frac{3}{\beta^2 B^2}\right) \exp\{-\frac{1}{2}(B-\beta)^2\} - \left(1 + \frac{3}{\beta B} + \frac{3}{\beta^2 B^2}\right) \exp\{-\frac{1}{2}(B+\beta)^2\} \right\} d\beta,$$

In the cases in which  $n_1$  is even, the probability of exceeding a given value  $B$  may be written

$$\int_B^\infty 2^{-\frac{1}{2}(n_1-2)} e^{-\frac{1}{2}\beta^2} \sum_{t=0}^{\infty} \frac{\beta^{2t}}{2^{2t} \cdot t! [\frac{1}{2}(n_1+2t-2)]!} x^{n_1+2t-1} e^{-\frac{1}{2}x^2} dx;$$

using the fact that when  $k$  is odd

$$\int_B^\infty \frac{x^k}{2^{\frac{1}{2}(k-1)} [\frac{1}{2}(k-1)]!} e^{-\frac{1}{2}x^2} dx = e^{-\frac{1}{2}B^2} \left\{ 1 + \frac{B^2}{2} + \frac{B^4}{2 \cdot 4} + \dots + \frac{B^{k-1}}{2 \cdot 4 \dots (k-1)} \right\},$$

the integral becomes

$$\sum_{t=0}^{\infty} e^{-\frac{1}{2}\beta^2} \frac{(\frac{1}{2}\beta^2)^t}{t!} + \sum_{u=0}^{\frac{1}{2}(n_1-2)} e^{-\frac{1}{2}B^2} \frac{(\frac{1}{2}B^2)^u}{u!},$$

involving only the terms of two Poisson Series with mean values  $\frac{1}{2}\beta^2$  and  $\frac{1}{2}B^2$ . If  $t$  and  $u$  be regarded as variates distributed independently in two such series,

the probability may be identified with the probability that  $u$  should not exceed  $t$  by  $\frac{1}{2}n_1$ , or more.

The distributions developed in this section are limiting forms appropriate to large samples, in which exact account is taken of the positive bias of small observed multiple correlations; they will provide at least an approximate treatment of those cases of great practical importance in which  $n_2$  does not exceed 100, and in which, therefore, the positive bias is prominent for observed values of  $R$  which are not small. The fact that sampling errors of the simple correlation coefficient have been successfully represented by a normal distribution by means of the transformation  $z = \tanh^{-1} r$ , suggests that pending fuller tests than are at present practicable, the transformation

$$B = \sqrt{n_2} \tanh^{-1} R, \quad \beta = \sqrt{n_2} \tanh^{-1} \rho,$$

will supply tests of significance of precision, sufficient for practical purposes, in the important region alluded to.

Table I (table of  $B$ ) shows the 5 per cent. points of these distributions, for

Table of 5 per cent. points of the distribution of  $B$ .

Values of $\beta_1$	Value of $n_1$						
	1.	2.	3.	4.	5.	6.	7.
0	1.9600	2.4477	2.7955	3.0802	3.3272	3.5485	3.7506
0.2	1.9985	2.4720	2.8140	3.0955	3.3405	3.5602	3.7613
0.4	2.1070	2.5419	2.8680	3.1405	3.3796	3.5951	3.7930
0.6	2.2654	2.6497	2.9533	3.2125	3.4426	3.6517	3.8445
0.8	2.4605	2.7855	3.0640	3.3076	3.5268	3.7278	3.9144
1.0	2.6461	2.9398	3.1941	3.4216	3.6291	3.8210	4.0005
1.2	2.8451	3.1059	3.3386	3.5505	3.7462	3.9289	4.1008
1.4	3.0449	3.2796	3.4935	3.6911	3.8756	4.0491	4.2134
1.6	3.2449	3.4584	3.6561	3.8408	4.0148	4.1796	4.3363
1.8	3.4449	3.6410	3.8246	3.9978	4.1620	4.3184	4.4681
2.0	3.6449	3.8263	3.9976	4.1604	4.3158	4.4645	4.6074
2.2	3.8449	4.0137	4.1743	4.3278	4.4750	4.6166	4.7531
2.4	4.0449	4.2027	4.3539	4.4990	4.6388	4.7738	4.9043
2.6	4.2449	4.3932	4.5359	4.6735	4.8065	4.9353	5.0603
2.8	4.4449	4.5847	4.7199	4.8506	4.9774	5.1006	5.2204
3.0	4.6449	4.7772	4.9055	5.0301	5.1512	5.2691	5.3840
3.2	4.8449	4.9705	5.0926	5.2115	5.3273	5.4404	5.5508
3.4	5.0449	5.1644	5.2809	5.3946	5.5056	5.6142	5.7204
3.6	5.2449	5.3589	5.4703	5.5792	5.6857	5.7901	5.8924
3.8	5.4449	5.4914	5.6006	5.7050	5.8075	5.9079	6.0065
4.0	5.6449	5.7493	5.8516	5.9521	6.0506	6.1475	6.2426
4.2	5.8449	5.9451	6.0434	6.1401	6.2351	6.3285	6.4204
4.4	6.0449	6.1412	6.2359	6.3290	6.4206	6.5109	6.5998
4.6	6.2449	6.3376	6.4288	6.5187	6.6072	6.6945	6.7805
4.8	6.4449	6.5342	6.6223	6.7091	6.7947	6.8792	6.9625
5.0	6.6449	6.7311	6.8162	6.9002	6.9831	7.0649	7.1457

values of  $\beta$  from 0 to 5 and of  $n_1$  from 1 to 7. The values tabulated are the values of B which will be exceeded by chance in 5 per cent. random trials, and which therefore give a presumption that  $\beta$  is really greater than the value postulated. Thus, when  $n_1 = 3$ , it may be seen at a glance that a value  $B = 5.7$  indicates that  $\beta$  probably exceeds 3.8.

For a great part of the labour of constructing this Table I am indebted to Mr. A. J. Page, I.C.S., whose assistance in my laboratory while on leave has thus enabled me to press forward with the theoretical investigation of the new distributions.

### 6. The Probability Integral.

For calculations involving finite probabilities of occurrence, including tests whether an observed R is or is not significantly discrepant from a hypothetical  $\rho$ , it is not the frequency element but its integral that is required. It is fortunate that the frequency distribution we have found when  $n_2$  is even leads to a probability integral of a tolerably simple form.

The frequency element

$$(1 - \rho^2)^{\frac{1}{2}(n_1+n_2)} \frac{n_1 + n_2 - 2}{2} ! \frac{(R^2)^{\frac{1}{2}(n_1-2)}}{[\frac{1}{2}(n_1-2)]!} \\ \times \frac{(1 - R^2)^{\frac{1}{2}(n_2-2)}}{[\frac{1}{2}(n_2-2)]!} F \left[ \frac{n_1 + n_2}{2}, \frac{n_1 + n_2}{2}, \frac{n_1}{2}, \rho^2 R^2 \right] d(R^2);$$

may be written

$$(1 - \rho^2)^{\frac{1}{2}(n_1+n_2)} \sum_{t=0}^{\infty} \frac{[\frac{1}{2}(n_1 + n_2 + 2t - 2)]!^2}{[\frac{1}{2}(n_1 + n_2 - 2)]! t!} \rho^{2t} \frac{(R^2)^{\frac{1}{2}(n_1+2t-2)}}{[\frac{1}{2}(n_1 + 2t - 2)]!} \\ \times \frac{(1 - R^2)^{\frac{1}{2}(n_2-2)}}{[\frac{1}{2}(n_2 - 2)]!} d(R^2);$$

but if  $n_2$  is even

$$\int_0^{R^2} \frac{n_1 + n_2 + 2t - 2}{2} ! \frac{(R^2)^{\frac{1}{2}(n_1+2t-2)}}{[\frac{1}{2}(n_1 + 2t - 2)]!} \cdot \frac{(1 - R^2)^{\frac{1}{2}(n_2-2)}}{[\frac{1}{2}(n_2 - 2)]!} d(R^2)$$

is

$$R^{n_1+2t} \left\{ 1 + \frac{n_1+2t}{2} (1 - R^2) + \frac{(n_1+2t)(n_1+2t+2)}{2 \cdot 4} (1 - R^2)^2 + \dots \right. \\ \left. + \frac{(n_1+2t) \dots (n_1+2t+n_2-4)}{2 \cdot 4 \dots (n_2-2)} (1 - R^2)^{\frac{1}{2}(n_2-2)} \right\}$$

or

$$\sum_{p=0}^{\frac{1}{2}(n_2-2)} \frac{(1 - R^2)^p}{p!} \cdot \frac{[\frac{1}{2}(n_1 + 2t + 2p - 2)]!}{[\frac{1}{2}(n_1 + 2t - 2)]!} (R^2)^{\frac{1}{2}(n_1+2t)}.$$

Again

$$\sum_{t=0}^{\infty} \frac{[\frac{1}{2}(n_1 + n_2 + 2t - 2)]!}{[\frac{1}{2}(n_1 + n_2 - 2)]! t!} \rho^{2t} \frac{[\frac{1}{2}(n_1 + 2t + 2p - 2)]!}{[\frac{1}{2}(n_1 + 2t - 2)]!} (R^2)^{\frac{1}{2}(n_1 + 2t)}$$

is

$$R^{n_1} \frac{[\frac{1}{2}(n_1 + 2p - 2)]!}{[\frac{1}{2}(n_1 - 2)]!} F\left(\frac{n_1 + n_2}{2}, \frac{n_1 + 2p}{2}, \frac{n_1}{2}, \rho^2 R^2\right)$$

or

$$\frac{[\frac{1}{2}(n_1 + 2p - 2)]!}{[\frac{1}{2}(n_1 - 2)]!} \cdot \frac{R^{n_1}}{(1 - \rho^2 R^2)^{\frac{1}{2}(n_1 + n_2 + 2p)}} F\left(-p, -\frac{n_2}{2}, \frac{n_1}{2}, \rho^2 R^2\right),$$

which terminates in  $p + 1$  terms, and is equivalent to

$$\frac{\frac{1}{2}(n_2)!}{[\frac{1}{2}(n_2 - 2p)]!} \cdot \frac{R^{n_1} (\rho^2 R^2)^p}{(1 - \rho^2 R^2)^{\frac{1}{2}(n_1 + n_2 + 2p)}} F\left(-p, -\frac{n_1 + 2p - 2}{2}, \frac{n_1 - 2p + 2}{2}, \frac{1}{\rho^2 R^2}\right),$$

or

$$\frac{(\frac{1}{2}n_2)!}{[\frac{1}{2}(n_2 - 2p)]!} \cdot \frac{R^{n_1} (-)^p}{(1 - \rho^2 R^2)^{\frac{1}{2}(n_1 + n_2)}} F\left(-p, \frac{n_1 + n_2}{2}, \frac{n_2 - 2p + 2}{2}, \frac{1}{1 - \rho^2 R^2}\right).$$

The probability integral, when  $n_2$  is even, may therefore be written in the forms

$$(1 - \rho^2)^{\frac{1}{2}(n_1 + n_2)} R^{n_1} \sum_{p=0}^{\frac{1}{2}(n_2 - 2)} \frac{[\frac{1}{2}(n_1 + 2p - 2)]!}{[\frac{1}{2}(n_1 - 2)]! p!} \frac{(1 - R^2)^p}{(1 - \rho^2 R^2)^{\frac{1}{2}(n_1 + n_2 + 2p)}} F\left(-p, -\frac{n_2}{2}, \frac{n_1}{2}, \rho^2 R^2\right),$$

or

$$\left(\frac{1 - \rho^2}{1 - \rho^2 R^2}\right)^{\frac{1}{2}(n_1 + n_2)} R^{n_1} \sum_{p=0}^{\frac{1}{2}(n_2 - 2)} (-)^p \frac{(\frac{1}{2}n_2)!}{[\frac{1}{2}(n_2 - 2p)]! p!} (1 - R^2)^p F\left(-p, \frac{n_1 + n_2}{2}, \frac{n_1 - 2p + 2}{2}, \frac{1}{1 - \rho^2 R^2}\right),$$

both of which terminate in  $\frac{n_2}{8}(n_2 + 2)$  elementary terms.

When  $n_2 = 2$ , we have the simple probability integral

$$\{(1 - \rho^2)/(1 - \rho^2 R^2)\}^{\frac{1}{2}(n_1 + 2)} R^{n_1};$$

when  $n_2 = 4$ , it becomes

$$\left(\frac{1 - \rho^2}{1 - \rho^2 R^2}\right)^{\frac{1}{2}(n_1 + 4)} \left\{ \frac{n_1 + 4}{2} \frac{1 - R^2}{1 - \rho^2 R^2} - (1 - 2R^2) \right\} R^{n_1},$$

and, when  $n_2 = 6$ ,

$$\left(\frac{1 - \rho^2}{1 - \rho^2 R^2}\right)^{\frac{1}{2}(n_1+6)} \left\{ \frac{(n_1+6)(n_1+8)}{2 \cdot 4} \left(\frac{1 - R^2}{1 - \rho^2 R^2}\right)^2 - \frac{n_1+6}{2} (2 - 3R^2) \frac{1 - R^2}{1 - \rho^2 R^2} + (1 - 3R^2 + 3R^4) \right\} R^{n_1}.$$

It should be observed that the coefficient of  $\{(1 - R^2)/(1 - \rho^2 R^2)\}^p$  is given by

$$\frac{[\frac{1}{2}(n_1 + n_2 + 2p - 2)]!}{[\frac{1}{2}(n_1 + n_2 - 2)]!} \frac{d^p}{p!^2} \left( \frac{(R^2 + x)^{\frac{1}{2}n_1} - R^{n_1}}{x} \right)$$

when  $x = -1$ .

### 7. *Extension of the Analysis of Variance.*

The distribution of the simple correlation coefficient, although one of the first sampling distributions to be determined with exactitude\*, has hitherto occupied a somewhat isolated position. For all the exact distributions of statistics since discovered have grouped themselves in a single system; they are all amenable to the same technical procedure known as the analysis of variance; and all may be reduced to an equivalent problem of the distribution of the difference of the logarithms of two independent estimates of variance, based respectively upon  $n_1$  and  $n_2$  degrees of freedom.

The distribution of such an estimate  $s_1^2$  derived from  $n_1$  degrees of freedom is given by

$$df = \frac{1}{[\frac{1}{2}(n_1 - 2)]!} t_1^{\frac{1}{2}(n_1-2)} e^{-t_1} dt_1, \text{ where } t_1 = \frac{n_1 s_1^2}{2\sigma^2},$$

and  $\sigma$  is the parameter of which  $s_1$  is the first estimate.

If, now,  $t_2 = n_2 s_2^2 / 2\sigma^2$ , and

$$z = \log s_1 - \log s_2,$$

it follows that

$$t_1 = (n_1/n_2) e^{2z} t_2,$$

and the simultaneous distribution

$$df = \frac{1}{[\frac{1}{2}(n_1 - 2)]!} t_1^{\frac{1}{2}(n_1-2)} e^{-t_1} dt_1 \cdot \frac{1}{[\frac{1}{2}(n_2 - 2)]!} t_2^{\frac{1}{2}(n_2-2)} e^{-t_2} dt_2$$

may be written

$$df = \frac{2}{[\frac{1}{2}(n_1 - 2)]! [\frac{1}{2}(n_2 - 2)]!} \left( \frac{n_1}{n_2} e^{2z} \right)^{\frac{1}{2}n_1} t_2^{\frac{1}{2}(n_1+n_2-2)} e^{t_2 \left(1 + \frac{n_1}{n_2} e^{2z}\right)} dt_2 dz;$$

\* Fisher, 'Biometrika,' vol. 10, p. 507 (1915).

this expression may be integrated with respect to  $t_2$  to yield the distribution of  $z$ , in the form

$$df = 2 \frac{[\frac{1}{2}(n_1 + n_2 - 2)]!}{[\frac{1}{2}(n_1 - 2)]! [\frac{1}{2}(n_2 - 2)]!} \cdot \frac{n_2^{\frac{1}{2}n_2} n_1^{\frac{1}{2}n_1} e^{n_1 z}}{(n_2 + n_1 e^{2z})^{\frac{1}{2}(n_1 + n_2)}} \cdot dz,$$

completely independent of the unknown variance.

By the insertion of the appropriate values of  $n_1$  and  $n_2$ , including the important bounding values of unity and infinity, the appropriate distribution of  $z$  for the analysis of variance is obtained. In the case, for example, of the multiple correlation coefficient drawn from uncorrelated material,  $n_1$  is equated to the number of independent variates,  $n_1 + n_2 + 1$  to the sample number, and  $2z$  to

$$\log (R^2/n_1) - \log (1 - R^2/n_2).$$

It was from the first obvious that this system was capable without formal modification of extension to the case in which  $s_1$  and  $s_2$  were estimates of two different parameters  $\sigma_1$  and  $\sigma_2$ ; for in such cases we have only to write  $\zeta = \log \sigma_1 - \log \sigma_2$ , and the distribution found above will be that appropriate to the variate  $z - \zeta$ .

The new system of distributions found for the multiple correlation coefficient derived from correlated material is not only a generalisation of that previously found\* for the simple correlation coefficient, but provides an extension of a different kind from that mentioned above to the analysis of variance. For the limiting distribution found in section 5 (distribution of B) may be interpreted as the distribution of the sum of the squares of  $n_1$  variates normally distributed with equal variance, but not with zero means as in all cases previously discussed.

To show this, let  $T = \frac{1}{2\sigma^2} \sum_{p=1}^{n_1} (x_p - a_p)^2$ , in which  $x_1, \dots, x_{n_1}$  are variates distributed independently about zero with common variance  $\sigma^2$ . Let  $\xi = S(ax)/\sigma S(a^2)$ , then  $\xi$  will be normally distributed about zero with unit variance, and if we write  $\frac{1}{2}\chi^2$  for  $T - \frac{1}{2}(\xi - \sqrt{S(a^2)/\sigma^2})^2$  or  $\frac{1}{2} \sum_1^{n_1} (x^2/\sigma^2) - \frac{1}{2}\xi^2$ , which is the sum of the squares of  $(n_1 - 1)$  quantities independently distributed about zero with unit variance, it appears that the distribution of  $\chi^2$  is of the familiar form

$$\frac{1}{[\frac{1}{2}(n_1 - 3)]!} (\frac{1}{2}\chi^2)^{\frac{1}{2}(n_1 - 3)} e^{-\frac{1}{2}\chi^2} d(\frac{1}{2}\chi^2),$$

and is independent of that of  $\xi$ , namely  $(2\pi)^{-\frac{1}{2}} e^{-\frac{1}{2}\xi^2} d\xi$ .

\* Fisher, 'Biometrika,' vol. 10, p. 507 (1915).

If, now,  $\beta$  is written for  $\sqrt{S(a^2)}/\sigma$  and  $x$  for  $\xi - \beta$ , it follows that

$$\frac{1}{2}\chi^2 = T - \frac{1}{2}x^2,$$

and as the same value of  $x^2$  is provided by the two values of  $\xi$ ,  $\beta \pm \sqrt{x^2}$ , the frequency element required from the distribution of  $\xi$  is

$$\frac{1}{\sqrt{2\pi}} \{e^{-\frac{1}{2}(\beta+x)^2} + e^{-\frac{1}{2}(\beta-x)^2}\} dx,$$

only positive values of  $x$  being now considered. Substituting for  $\chi^2$  in terms of  $T$  and  $x$ , the frequency distribution of the latter two variates will be given by

$$df = \frac{1}{[\frac{1}{2}(n_1-3)]!} (T - \frac{1}{2}x^2)^{\frac{1}{2}(n_1-3)} dT \cdot \frac{2}{\sqrt{2\pi}} e^{-\frac{1}{2}\beta^2} e^{-T} \cosh(\beta x) \cdot dx.$$

For a given value of  $T$ , the variate  $x$  cannot exceed  $\sqrt{2T}$ , and the random sampling distribution of  $T$  is therefore found by integrating between 0 and  $\sqrt{2T}$ . Expanding the hyperbolic cosine in powers of  $x$ , and integrating term by term, since

$$\begin{aligned} \int_0^{\sqrt{2T}} (T - \frac{1}{2}x^2)^{\frac{1}{2}(n_1-3)} \frac{x^{2p} \beta^{2p}}{(2p)!} dx \\ = \frac{[\frac{1}{2}(2p-1)]! [\frac{1}{2}(n_1-3)]!}{[\frac{1}{2}(n_1+2p-2)]!} T^{\frac{1}{2}(n_1+2p-2)} 2^{\frac{1}{2}(2p-1)} \frac{\beta^{2p}}{(2p)!}, \end{aligned}$$

we have the distribution of  $T$  in the form.

$$df = e^{-\frac{1}{2}\beta^2} e^{-T} \sum_{p=0}^{\infty} \frac{T^{\frac{1}{2}(n_1+2p-2)} \beta^{2p}}{[\frac{1}{2}(n_1+2p-2)]! 2^p \cdot p!} dT.$$

or

$$df = e^{-\frac{1}{2}\beta^2} \frac{T^{\frac{1}{2}(n_1-2)}}{[\frac{1}{2}(n_1-2)]!} e^{-T} \left\{ 1 + \frac{1}{n_1} (T\beta^2) + \frac{1}{n_1(n_1+2)} \frac{(T\beta^2)^2}{2!} + \dots \right\} dT,$$

which is the B-distribution of section 5 if  $T$  is equated to  $\frac{1}{2}B^2$ .

This interpretation of the distribution previously obtained adds greatly to its importance, for it is seen to replace the  $\chi^2$  distribution of the analysis of variance for cases in which the sum of squares corresponding to  $n_1$  degrees of freedom is derived theoretically for non-central deviations with fixed central displacements. This will be similar to, but not identical with, the case of the  $n_1$  degrees of freedom in multiple correlation in its proper form; for although these are non-central, the displacements will depend on the variation in the sample of the independent variates, and this will vary from sample to sample. In many cases, however, such as the dependence of weather upon the position and altitudes of a number of fixed meteorological stations, we are not interested

in the effects of possible variations in the positions of the stations, but solely in the possible variations of the weather at these spots. In fact, the problem of practical importance is often that in which the central displacements are constant, and although it may be urged, rightly enough, that to such cases the purely empirical concept of multiple correlation is not the most appropriate approach, yet it remains true that of the practical applications of multiple correlation methods many are of this kind.

The direct extension of the analysis of variance for non-central squares may be completed by writing

$$df = \frac{1}{[\frac{1}{2}(n_2-2)]!} t_2^{\frac{1}{2}(n_2-2)} e^{-t} dt_2 \quad \text{and} \quad \frac{T}{t_2} = \frac{R^2}{1-R^2} = \frac{n_1}{n_2} e^{2z},$$

then, if, in spite of the caution above, we choose to express our results in terms of  $R$ ,

$$t_2 = \frac{1-R^2}{R^2} T, \quad dt_2 = -\frac{1-R^2}{R^2} T \frac{d(R^2)}{R^2(1-R^2)}, \quad t_2 + T = \frac{T}{R^2},$$

and the distribution of  $R$  is found by integrating with respect to  $T$  from 0 to  $\infty$ , the expression

$$\frac{1}{[\frac{1}{2}(n_2-2)]!} e^{-\frac{1}{2}\beta^2} e^{-T/R^2} \left( \frac{1-R^2}{R^2} \right)^{\frac{1}{2}n_2} \frac{dR^2}{R^2(1-R^2)} \sum_{p=0}^{\infty} \frac{T^{\frac{1}{2}(n_1+n_2+2p-2)} \beta^{2p}}{[\frac{1}{2}(n_1+2p-2)]! \, 2^p \cdot p!} dT,$$

a process which yields

$$df = (R^2)^{\frac{1}{2}(n_1-2)} \frac{(1-R^2)^{\frac{1}{2}(n_2-2)}}{[\frac{1}{2}(n_1-2)]!} e^{-\frac{1}{2}\beta^2} \sum_{p=0}^{\infty} \frac{[\frac{1}{2}(n_1+n_2+2p-2)]! (R^2\beta^2)^p}{[\frac{1}{2}(n_1+2p-2)]! \, 2^p \cdot p!} d(R^2).$$

or

$$df = \frac{[\frac{1}{2}(n_1+n_2-2)]!}{[\frac{1}{2}(n_1-2)]! [\frac{1}{2}(n_2-2)]!} (R^2)^{\frac{1}{2}(n_1-2)} (1-R^2)^{\frac{1}{2}(n_2-2)} e^{-\frac{1}{2}\beta^2} \left\{ 1 + \frac{n_1+n_2}{n_1 \cdot 1!} \frac{R^2\beta^2}{2} \right. \\ \left. + \frac{(n_1+n_2)(n_1+n_2+2)}{n_1(n_1+2) \cdot 2!} \left( \frac{R^2\beta^2}{2} \right)^2 + \dots \right\} d(R^2), \quad (C)$$

a third general distribution of this interesting group.

Although it will not be possible within the limits of this paper to give an account of the properties of the distribution of Type (C), beyond indicating their analogy with those of Type (A), it should not be overlooked that in the problems in which the multiple correlation coefficient is actually employed, distributions of Type (C) will be, owing to the absence or irrelevance of sampling variation in the variances of the independent variates, of at least as frequent occurrence as those of Type (A).

A typical example of the distinction here drawn is provided by the correlation ratio. If corresponding to any value  $x$  of the independent variate a number of values  $n_x$  of the dependent variate  $y$  is observed, then the correlation ratio  $\eta^2$  of  $y$  on  $x$  is defined by the relation

$$\frac{\eta^2}{1 - \eta^2} = \frac{S \{n_x (\bar{y}_x - \bar{y})^2\}}{S (y - \bar{y}_x)^2},$$

in which  $\bar{y}_x$  is the mean of  $y$  in any array, and  $\bar{y}$  is the general mean; the variance in all arrays is supposed equal, and the summation in the numerator is applied to the several arrays, while that in the denominator is applied to the whole of the individual observations. In most practical cases the idea of a sampling distribution of  $\eta^2$  can only be given a definite meaning by supposing the number  $n_x$  in each array to be the same for all samples. In such a case the distribution of  $\eta^2$  will be that of  $R^2$  in distribution (C), with  $n_1$  equal to one less than the number of arrays, and  $n_1 + n_2 + 1$  equal to the total number of observations. If, however, the numbers  $n_x$  be regarded as subject to sampling variations, then the distribution (A) may be used, and will be exact, apart from grouping errors, if the expectations of  $y$  for the values of  $x$  in the sampled population are normally distributed.

#### *Summary.*

By an appropriate linear transformation of the independent variates it may be shown that the sampling distribution of the multiple correlation coefficient does not depend on the whole matrix of correlations between these variates, but solely upon the multiple correlation in the population sampled.

The actual distribution (A) may then easily be obtained by similar methods to those by which the distribution of the simple correlation coefficient has been obtained.

The frequency function involves a hypergeometric function of  $\rho^2 R^2$  which is a rational function when  $n_1$  and  $n_2$  are both even, algebraic when  $n_2$  only is even, and reducible to circular functions when  $n_1$  and  $n_2$  are both odd.

The case of large samples yields a series of distributions (B) of great interest, involving Bessel functions, which connect the  $\chi^2$  distributions with the Gaussian, and are intimately related to a double Poisson summation. Owing to the practical importance of this limiting form a table of its 5 per cent. points is given up to seven independent variates.

When  $n_2$  is even, the probability integral of the general distribution is expressible in finite terms which are developed in section 6.

The (B) distribution of Section 5 replaces the  $\chi^2$  distribution in the analysis of variance if the squares summed are non-central. An analysis of variance so extended leads to a third group of distributions (C), closely related to (A), and tending like it to a common limit (B). The distinction between (A) and (C) arises from the fact that in cases proper to the multiple correlation the central displacements will vary from sample to sample owing to variations in the second order moment coefficients of the independent variates, and for such cases (A) is the correct distribution. The type (C), however, is of frequent occurrence owing to the absence or irrelevance of such variation.



# *Tests of Significance in Harmonic Analysis.*

By R. A. FISHER, F.R.S.

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## 1. *Schuster's Test.*

If a series  $u_1, u_2, \dots, u_{2n+1}$  constitute a random sample from a normally distributed population, then any linear function

$$A = \sum_1^{2n+1} (a_r u_r)$$

will also be normally distributed ; moreover its mean will be zero if  $S(a_r) = 0$ , and its variance will be equal to that of the original population if

$$S(a_r^2) = 1.$$

Any other linear function

$$B = \sum_1^{2n+1} (b_r u_r)$$

will be distributed independently of the first if

$$S(a_r b_r) = 0,$$

and in this case the sum of the squares,

$$x = A^2 + B^2,$$

will be distributed so that the chance of exceeding any particular value of  $x$  is

$$e^{-\frac{x}{c}},$$

where  $c$  is the mean value of  $x$ , equal to twice the variance of the population sampled.

This proposition, which gives the  $\chi^2$  distribution for the particular case  $n = 2$ , is the basis of Schuster's test of the significance of any particular term in the harmonic analysis of a series. For the coefficients

$$a_r = \sqrt{\frac{2}{2n+1}} \cos \frac{2\pi pr}{2n+1},$$

$$b_r = \sqrt{\frac{2}{2n+1}} \sin \frac{2\pi pr}{2n+1},$$

fulfil the necessary conditions for all integral values of  $p$ . Values of  $p$  from 1 to  $n$  give independently distributed values of  $x$  and, if the variance of the

population were known *a priori*, the test would be rigorous for any one of these chosen in advance.

### 2. Allowance for Selection of the Largest Term.

The practice of picking out the larger values of  $x$ , not in advance, but by reason of their exceptional magnitude, requires, as Sir Gilbert Walker has shown, an important modification of the test of significance. For, if we wish to test the significance of the largest observed value of  $x$ , we must compare the value observed with the sampling distribution of the largest of  $n$  independent values, and not with that of any one value chosen in advance. If  $P$  stand for the probability which we adopt, as sufficiently small to be used as a criterion of significance, the corresponding value of  $x$  will be given by

$$e^{-\frac{x}{c}} = P,$$

for any particular term, but if  $x$  is chosen to be the largest of  $n$  independent values, it is necessary that the probability should be  $1 - P$  that all the  $n$  values shall be less than  $x$ , so that

$$\left(1 - e^{-\frac{x}{c}}\right)^n = 1 - P$$

is the equation which determines the least value of  $x$  to be judged significant. This is the criterion derived by Walker.

### 3. Allowance for the Sampling Error of the Estimated Variance.

In the practical application of this criterion, when  $c$  is not known *a priori*, it is necessary to substitute for  $c$  an estimate of it derived from the data, and, for an exact test, to take into account the sampling error of this estimate. The estimate of  $c$  will necessarily be based on the variance observed in the original sample, or, what comes to the same thing, on the average value of  $x$  for the  $n$  possible periods; and, whether we take, as our actual estimate, the average of all the  $n$  values, or the average of the  $(n - 1)$  values other than that to be tested, all that is required for an exact solution, in either case, is the frequency distribution of the largest of  $n$  values of  $x$ , expressed as a fraction of the total of the sample of  $n$  of which it is the largest member.

If  $x_1, \dots, x_n$  are the co-ordinates of a point in Euclidian space of  $n$  dimensions, the simultaneous distribution of the  $n$  values will be represented by a density function

$$e^{-\frac{1}{c}(x_1 + x_2 + \dots + x_n)}$$

which is constant over plane finite regions of  $n - 1$  dimensions, bounded by the  $n$ -surfaces

$$x_r = 0$$

in the form of a generalised tetrahedron. In every such region, the distribution of the ratio of the largest co-ordinate to the sum of all co-ordinates will be the same, and, since the density is constant over each such region, the distribution is to be found merely from the elements of generalised volume, into which the region is divided for fixed values of the ratio. Any particular co-ordinate, *e.g.*,  $x_1$ , will be the greatest in one  $n$ th of the whole region, this fraction being bounded on the one hand by the loci, at which it ceases to be greatest.

$$x_1 = x_2, \quad x_1 = x_3, \quad \dots, \quad x_1 = x_n,$$

and, on the other, by the boundaries,

$$x_2 = 0, \quad x_3 = 0, \quad \dots, \quad x_n = 0;$$

within this region it is required to find the distribution of the ratio

$$g = \frac{x_1}{x_1 + x_2 + \dots + x_n}.$$

#### 4. The Discontinuities of the Distribution.

The distribution defined geometrically by the dissection of a generalised tetrahedron exhibits a number of discontinuities; the linear regions which constitute its boundary intersect  $n - 1$  at a time at the sets of points typified by

$$\begin{aligned} x_1 = x_2 = x_3 = \dots &= x_n \\ x_2 = 0, \quad x_1 = x_3 = \dots &= x_n \\ x_2 = x_3 = 0, \quad x_1 = x_4 = \dots &= x_n \\ \dots &\dots \\ x_2 = x_3 = \dots &= x_n = 0, \end{aligned}$$

at which it is evident that the values of  $g$  are

$$\frac{1}{n}, \quad \frac{1}{n-1}, \quad \dots, \quad \frac{1}{2}, \quad 1,$$

representing in succession, the centre of the generalised tetrahedron, the centres of all its bounding faces, of successively lower dimensions, which meet in the point,  $g = 1$ , the middle points of the edges running from this point,

and finally the limiting point,  $g = 1$ , itself. Hence  $g$  is distributed over the range from  $1/n$  to 1; and for an exact test of significance we require to know the probability with which any particular value between these limits is exceeded.

### 5. The Exact Distribution.

A point about the distribution which greatly facilitates the solution, is that within the region between any two discontinuities the probability integral of the distribution is merely a polynomial in  $g$  of degree  $n - 1$ . For the boundaries of any region,  $g = g_0$ , change the magnitude of their elements continuously at rates determined by the magnitude of their boundaries, and so on down to the bounding edges, the lengths of which are linear functions of  $g$ ; consequently the probability integral is in each region a polynomial of degree  $n - 1$ , but from region to region the  $(n - 1)$ th differential coefficient with respect to  $g$  changes discontinuously.

We may therefore represent the probability integral by the form

$$P = \alpha_1(1 - g)^{n-1} + \alpha_2(1 - 2g)^{n-1} + \dots + \alpha_n(1 - ng)^{n-1},$$

in which as many terms are to be taken as have positive quantities within the brackets. The last term is therefore included for no possible value of  $g$ , but is written above in order to utilise the condition that when  $g < 1/n$  the probability integral shall be unity. This condition is sufficient to determine the  $n$  coefficients by equation of the coefficients of  $g^0, g^1, \dots, g^{n-1}$ .

To determine their actual values let

$$f = -1 + \alpha_1 t + \alpha_2 t^2 + \dots + \alpha_n t^n,$$

then the equations of the successive coefficients give

$$f = 0, \quad \left(t \frac{d}{dt}\right) f = 0, \quad \dots, \quad \left(t \frac{d}{dt}\right)^{n-1} f = 0,$$

at the values  $t = 1$ . These are evidently equivalent to

$$f = 0, \quad \frac{d}{dt} f = 0, \quad \dots, \quad \frac{d^{n-1}}{dt^{n-1}} f = 0,$$

for the same value, so that  $f$ , being of degree  $n$ , must be a numerical multiple of  $(t - 1)^n$ , or, in view of its first term,

$$f = -(1 - t)^n.$$

We have therefore the probability integral in the form

$$P = n(1-g)^{n-1} - \frac{n(n-1)}{2}(1-2g)^{n-1} + \dots + (-)^{k-1} \frac{n!}{k!(n-k)!} (1-kg)^{n-1},$$

where  $k$  is the greatest integer less than  $1/g$ .

#### 6. Summary and Table of 5 per cent. Values.

A practical convenience of the form which has been obtained for the probability integral, is that for small values of  $P$ , such as are needed in tests of significance, the magnitude of the successive terms decreases very rapidly, so that even when, as at the 5 per cent. point for  $n = 50$ , as many as seven terms exist, very high precision is obtained from the first three terms only. Indeed the first term alone gives a very satisfactory approximate test of significance. The first term has, moreover, a simple meaning in relation to a related statistical problem. There are, in fact four related distributions each of which is the appropriate solution of one of four problems.

(I) The distribution of any one harmonic term obtained from a random sample of numbers drawn from a population of known variance. Schuster's solution of this is given by the distribution of the form

$$P = e^{-x}. \quad (1)$$

(II) The distribution of the largest of the  $n$  harmonic terms obtained from a similar sample; for this we have Walker's solution

$$P = 1 - (1 - e^{-x})^n. \quad (2)$$

(III) We may ask what is the distribution of any one harmonic term as a fraction of the total (or mean) of the terms obtained from the same sample; here there is no restriction that our term should be the largest, and all points within the generalised tetrahedron are available, so that

$$P = (1 - g)^{n-1} \quad (3)$$

where  $g$  is the chosen term expressed as a fraction of the whole.

(IV) Finally the probability that the largest of the  $n$  terms should exceed  $g$  is, so long as this probability is small, naturally not far from  $n$  times the value given by (3), and has been shown to be exactly

$$P = n(1-g)^{n-1} - \dots + (-)^{n-1} \frac{n!}{k!(n-k)!} (1-kg)^{n-1}, \quad (4)$$

where  $k$  is the largest integer less than  $1/g$ .

How good an approximation is obtained by using the first term only, is shown by the following table giving the 5 per cent. values of  $g$  for values of  $n$  from 5 to 50 in a parallel column with those obtained by ignoring all terms after the first.

$n$ .	$g$ (by exact formula).	$g$ (by first term only).
5	0·68377	0·68377
10	0·44495	0·44495
15	0·33462	0·33463
20	0·27040	0·27046
25	0·22805	0·22813
30	0·19784	0·19794
35	0·17513	0·17525
40	0·15738	0·15752
45	0·14310	0·14324
50	0·13135	0·13149

This table can be used directly in testing significance ; the 5 per cent. point is the lowest level of significance likely to be wanted, and for higher levels, such as the 1 per cent. point, the first term will provide an even closer approximation. The method of section 5 should be useful in many distribution problems involving points of discontinuity.

The value of  $g$  may in all cases be very easily obtained. If all the Fourier submultiples have been worked out, it is, as already defined,

$$x_1 + x_2 + \dots + x_n$$

The denominator of this expression is, however, merely

$$\sum_{r=1}^{2n+1} (u_r - \bar{u})^2.$$

In the case where the number of observations in the series is even,  $(2n + 2)$ , we need still only consider the  $n$  complete harmonic terms, and can obtain their sum as

$$\sum_{r=1}^{2n+2} (u_r - \bar{u})^2 - \frac{(u_1 - u_2 + u_3 - \dots - u_{2n+2})^2}{2n + 2}.$$

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*Limiting forms of the frequency distribution of the largest or smallest member of a sample.* By R. A. FISHER, Sc.D., Gonville and Caius College, and L. H. C. TIPPETT, M.Sc.

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### 1. *Introductory.*

In a previous paper on the subject of the distribution of the largest member of a sample from a normal population, one of the authors has given constants involving the first four moments for samples up to 1000. In this paper, possible limiting forms of such distributions in general are discussed. It will appear that a particular group of distributions provides the limiting distributions in all cases, and that the case derived from the normal curve is peculiar for the extreme slowness with which the limiting form is approached.

### 2. *The possible limiting forms deduced from the functional relation which they must satisfy.*

Since the extreme member of a sample of  $mn$  may be regarded as the extreme member of a sample of  $n$  of the extreme members of samples of  $m$ , and since, if a limiting form exist, both of these distributions will tend to the limiting form as  $m$  is increased indefinitely, it follows that the limiting distribution must be such that the extreme member of a sample of  $n$  from such a distribution has itself a similar distribution.

If  $P$  is the probability of an observation being less than  $x$ , the probability that the greatest of a sample of  $n$  is less than  $x$  is  $P^n$ , consequently in the limiting distributions we have the functional equation

$$P^n(x) = P(a_n x + b_n);$$

the solutions of this functional equation will give all the possible limiting forms.

If  $a$  is not equal to unity, then

$$x = ax + b,$$

when

$$x = \frac{b}{1-a},$$

and at this point

$$P^n = P,$$

$$P = 0 \text{ or } 1,$$

consequently the solutions fall into three classes:

- |                            |                        |
|----------------------------|------------------------|
| I. $a = 1,$                | $P^n(x) = P(x + b_n),$ |
| II. $P = 0$ when $x = 0,$  | $P^n(x) = P(a_n x),$   |
| III. $P = 1$ when $x = 0,$ | $P^n(x) = P(a_n x).$   |

I. If  $P^n(x) = P(x + b_n)$ ,  
 then  $n \log P(x) = \log P(x + b_n)$ ,  
 and  $\log n + \log(-\log P(x)) = \log(-\log P(x + b_n))$ ;  
 therefore the expression  $\log(-\log P(x)) - \frac{x \log n}{b_n}$  is constant, or  
 periodic, with period  $b_n$ .

Now for all values of  $m$  and  $n$

$$b_{mn} = b_m + b_n,$$

and if  $b_n$  is an analytic function of  $n$ , a supposition which excludes the periodic solution,

$$nb'_{mn} = b'_m, \quad nb'_{mn} = b'_n,$$

whence

$$mb'_m = nb'_n,$$

or

$$b'_n = \frac{c}{n},$$

and

$$b_n = c \log n + d, \text{ where } c \text{ and } d \text{ are constants.}$$

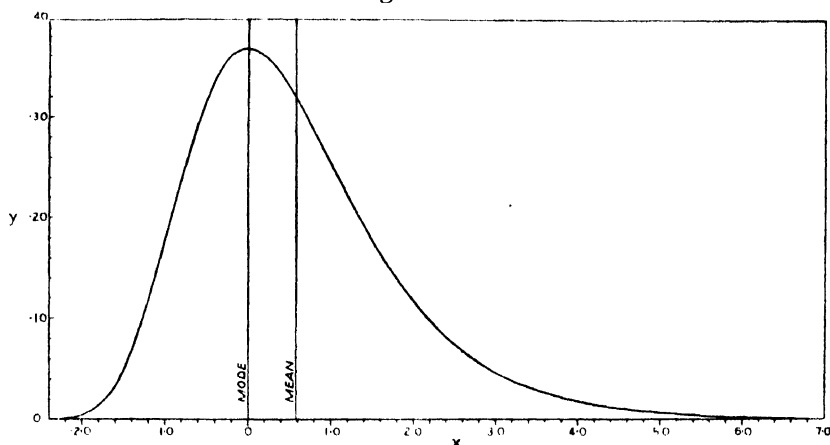


Fig. 1. Distribution  $p = e^{-e^{-x}}$ , or  $dp = e^{x-e^{-x}} dx$  represented by the curve  $y = e^{x-e^{-x}}$ .

Putting  $n = 1$ , it appears that

$$d = 0.$$

Hence  $\log(-\log P_x) = \frac{x}{c} + \text{constant}$ ,

or, the limiting form is that of  $-\log(-\log P_x) = x$ , for  $c$  must be negative since  $x$  is assumed to increase with  $P$ . The distribution of the greatest of a sample of  $n$  from this distribution is

$$-\log(-\log P_x) = x - \log n,$$

the distribution being merely shifted, without change of size or form, through a distance  $\log n$ . The curve is shown in Fig. 1.

II and III. If  $P^n(x) = P(a_n x)$ ,

$$a_{mn} = a_m a_n,$$

and, if  $a$  is an analytic function of  $n$ ,

$$ma'_{mn} = a_m a'_n, \quad na'_{nm} = a'_m a_n,$$

whence

$$\frac{a'_n}{a_n} = \frac{-1}{kn},$$

of which the solution is

$$\log a_n = \frac{-1}{k} \log n, \quad a_n = n^{-1/k},$$

since  $a = 1$  when  $n = 1$ .

Now  $\log(-\log P(x))$  is increased by  $\log n$  when  $\log x$  is increased by  $\log a_n$ , so that, excluding as before the periodic solution,

$$\log(-\log P(x)) - \frac{\log n \log x}{\log a_n}$$

must be constant. This gives

$$\log(-\log P(x)) = -k(\log x + c)$$

or

$$-\log P(x) = (Ax)^{-k}.$$

If  $P = 0$  when  $x = 0$ ,  $k$  will be positive (II).

The form of the curve is then that of

$$P = e^{-x^{-k}},$$

$$dP = \frac{k}{x^{k+1}} e^{-x^{-k}} dx, \text{ where } k \text{ is positive.}$$

If  $P = 1$  when  $x = 0$ ,  $k$  will be negative and all possible values of  $x$  will be negative; in this case (III) the form of the curve is given by

$$-\log P = (-x)^k, \text{ where } k \text{ is positive,}$$

$$P = e^{-(-x)^k},$$

$$dP = k(-x)^{k-1} e^{-(-x)^k} dx.$$

The only possible limiting curves are therefore:

I.

$$dP = e^{-x - c^{-x}} dx,$$

in which the effect of selecting the greatest value of a sample of  $n$  is merely to shift the curve, without affecting its scale, through a distance  $\log n$ .

II.

$$dP = \frac{k}{x^{k+1}} e^{-x^{-k}} dx,$$

in which the effect of selection is to increase the scale of the curve by the factor  $n^{1/k}$ , maintaining the terminus  $x = 0$  unchanged.

### III.

$$dP = k(-x)^{k-1} e^{-(-x)^k} dx,$$

in which the effect of selection is to decrease the scale of the curve by the factor  $n^{-1/k}$ , while maintaining the terminus  $x=0$  unchanged. In this case alone will the selected curve increase materially in accuracy as selection is increased; the weight of an observation, from curves of constant form, will be inversely proportional to the square of the scale, and will be proportional to  $n^{2/k}$ . The accuracy of the extreme observation will therefore increase more rapidly than that of, for example, the mean, if  $k$  is less than 2.

3. *The limiting form appropriate to any particular frequency distribution.*

If in any frequency distribution  $p$  is the probability of an observation being less than  $x$ , and if as  $p \rightarrow 1$  the quantity

$$(1-p)x^k$$

tends to a finite limit,  $a^k$ , then it is evident that  $P=p^n$  will have the form

$$P = e^{-na^k x^{-k}}$$

in the limit for large samples of  $n$ .

Since, for any two values of  $P$  other than 0 and 1, the values of  $x$  as  $n$  tends to infinity tend to the finite ratio of the values of

$$(-\log P)^{-1/k}$$

the limiting form of the distribution will be the same if

$$1-p = x^{-k} \phi(x),$$

where the range of  $\log \phi$ , for any finite range of  $\log x$ , tends to zero as  $x$  tends to infinity.

The scale of the distribution for the greatest of  $n$ , measured by  $an^{1/k}$ , will in such cases approach the limit

$$(\phi n)^{1/k},$$

where the argument of  $\phi$  is given by the equation

$$x^k = n\phi(x).$$

Equally, for any frequency distribution for which

$$(1-p)e^{x/c}$$

tends to a finite limit  $A$  as  $p$  tends to unity, the limiting forms of the distribution of the largest of a sample of  $n$  will be given by

$$P = e^{-nAe^{-x/c}}.$$

Since, in this case, for any two values of  $P$  other than 0 and 1, the difference of the two values of  $x/c$  tends to a constant value, the limiting form of distribution will be the same if

$$1 - p = e^{-x/c} \phi(x),$$

when the range of  $\log \phi$  in any finite range of  $x/c$  tends to zero as  $x$  tends to infinity. Thus, if  $c$  is constant,  $\phi(x)$  may contain factors such as  $x^t$ . The location of the distribution, given by

$$\frac{x}{c} = \log(nA),$$

will then, as the limiting form is approached, change as  $c \log(n\phi)$ , in which the argument of  $\phi$  is given by the equation

$$x = c \log(n\phi(x)).$$

The case in which  $c$  is constant does not exhaust the applications of this limiting form, for whatever function  $1 - p$  may be of  $x$ , if we write

$$\frac{1}{c} = - \frac{d}{dx} \log(1 - p),$$

then, if the range of  $\log(1 - p) + x/c$  from  $x = \xi$  to  $x = \xi + ct$ , tends to zero, as  $x$  tends to infinity, for all real values of  $t$ , then will the same limiting form be valid.

For example, let

$$1 - p = e^{-x^r},$$

then

$$c = \frac{1}{r \xi^{r-1}},$$

and

$$\frac{ct}{\xi} = \frac{t}{r \xi^r},$$

which tends to zero, if  $r$  is positive, for all values of  $t$ .

But

$$\begin{aligned} \log(1 - p) + x/c &= r x \xi^{r-1} - x^r \\ &= (r-1) \xi^r - \frac{r(r-1)}{2} \xi^{r-2} c^2 t^2 + \text{smaller terms;} \end{aligned}$$

the range will therefore tend to zero, for

$$\frac{r(r-1)}{2} \xi^{r-2} c^2 t^2 = \frac{r-1}{2r} \frac{t^2}{\xi^r},$$

which tends to zero, for all values of  $t$ , if  $r$  is positive.

The parameter  $c$ , which measures the scale of the distribution, will increase if  $r < 1$ , and decrease if  $r > 1$ , while the location of the mode as the limiting form is approached is given in general by

$$P = e^{-1},$$

or

$$n(1-p) = 1.$$

Again, for the normal curve with unit standard deviation

$$1-p = \frac{1}{\sqrt{2\pi}} e^{-\frac{1}{2}x^2} x^{-1} (1-X),$$

where  $X$  tends to zero as  $x$  tends to infinity,

$$\frac{1}{c} = \xi + \frac{1}{\xi} - X,$$

$$\begin{aligned} \log(1-p) + x/c &= -\frac{1}{2}x^2 - \log x + x\xi + 1 - \frac{1}{2}\log(2\pi) - X \\ &= +\frac{1}{2}\xi^2 - \log \xi + 1 - \frac{1}{2}\log(2\pi) - X, \end{aligned}$$

where  $X$  vanishes as  $x \rightarrow \infty$ , at all values of  $x$  from  $\xi$  to  $\xi + ct$ .

For sufficiently large samples of  $n$  from a normal curve, the distribution of the largest of the sample will be centred about a mode  $m$  given by

$$e^{\frac{1}{2}m^2} m\sqrt{2\pi} = n,$$

with scale given by

$$c = \frac{m}{m^2 + 1}.$$

4. *The approach of the distribution of the greatest of a normal sample to its final form.*

The final form for the largest of a normal sample has been shown to be given by

$$P = e^{-e^{-x/c}},$$

where  $c$  diminishes to zero as the sample increases, in such a way that to the degree of approximation required in very large samples

$$c = \frac{m}{m^2 + 1}$$

and

$$e^{\frac{1}{2}m^2} m\sqrt{2\pi} = n.$$

Since for any finite value of  $m$ , however large,  $c$  will still be diminishing as  $n$  increases, the case has an analogy at any stage with the distribution derived from

$$p = e^{-(x)^k},$$

in which also the scale diminishes as  $n$  increases. This analogy

may be utilised by equating the rate of change of the scale with increasing  $n$  in the two cases.

Now, for  $P = e^{-n(-x)^k}$ ,  
 we have  $dP = kn(-x)^{k-1} e^{-n(-x)^k} dx$ ,  
 so that the logarithm of the ordinate at any point is  
 $(k-1) \log(-x) - n(-x)^k + \text{constant}$ ,  
 giving as equation for the mode,  $m$ ,

$$(-x)^k = \frac{k-1}{nk},$$

whence 
$$\frac{d \log(-x)}{d(\log n)} = -\frac{1}{k}.$$

But for the normal curve

$$\frac{d \log c}{d \log n} = \frac{d \log c}{dm} \frac{dm}{d \log n} = -\frac{m^2 - 1}{(m^2 + 1)^2}.$$

Hence the distribution in which

$$\frac{m^2 - 1}{(m^2 + 1)^2} = \frac{1}{k} = h$$

should provide a penultimate form of approximation, which will duly tend to the ultimate form as  $h$  tends to zero.

### 5. *The moments of the ultimate and penultimate forms.*

The moments of the ultimate form

$$dP = e^{-x - e^{-x}} dx$$

may be found most directly from the generating function of the semi-invariants

$$K = \log M,$$

where

$$M = \int_{-\infty}^{\infty} e^{tz} dP.$$

For, writing  $z$  for  $e^{-x}$ ,

$$M = \int_0^{\infty} z^{-t} e^{-z} dz = (-t)!$$

and 
$$K = \log M = \gamma t + \frac{\pi^2}{6} \frac{t^2}{2!} + \frac{t^3}{3!} \left( -\frac{d^3}{dz^3} \log x! \right)_{x=0} + \dots,$$

whence it follows that the distance of the mean from the mode is

$$\mu_1' = \gamma = .577215665,$$

the variance is 
$$\mu_2 = \frac{\pi^2}{6} = 1.64493407,$$

the third moment is

$$\mu_3 = 2 \left\{ 1 + \frac{1}{2^3} + \frac{1}{3^3} + \dots \right\} = 2.40411381,$$

while the fourth moment is given by

$$\mu_4 - 3\mu_2^2 = 6 \left\{ 1 + \frac{1}{2^4} + \frac{1}{3^4} + \dots \right\} = \frac{\pi^4}{15} = 6.4939394.$$

Consequently, for sufficiently large samples we shall have

$$\text{Mean} - \text{Mode} = \gamma c = \frac{\gamma m}{m^2 + 1},$$

$$\text{Variance} = \frac{\pi^2}{6} c^2,$$

$$\beta_1 = 1.2985676,$$

$$\beta_2 = 5.4.$$

For the penultimate form

$$dP = k(-x)^{k-1} e^{-(-x)^k} dx,$$

writing

$$-x = t^{\frac{1}{k}} = t^k,$$

we have

$$dP = -e^{-t} dt,$$

and

$$\mu_r' = (-)^r (-x)^r dP = (-)^r t^{kr} dP = (-)^r (kr)!;$$

also the mode is given by

$$-x = (1-h)^{\frac{1}{k}}.$$

Hence we have as penultimate formulae

$$\text{Mean} - \text{Mode} = \frac{c}{h} \{(1-h)^h - h!\},$$

$$\text{Variance} = \frac{c^2}{h^2} \{(2h)! - (h!)^2\},$$

together with  $\beta_1$  and  $\beta_2$  expressed in terms of  $h$  only.

The extreme slowness with which the ultimate form is approached is well shown by the fact that even for enormous samples the penultimate form is still materially different in its  $\beta$  coefficients. The following tables show, for different values of  $h$ , the corresponding values of  $m$  and  $n$ , and, in parallel columns, the distance of the mean from the mode, the variance and the  $\beta$  coefficients. It will be observed that even for samples of nearly a billion the penultimate form is still considerably different from the ultimate form. The appropriateness of the penultimate form for samples of 1000 downwards can be tested from the results given in a previous paper\*, using for  $m$  the value of  $x$  for which  $p = 1/n$ , and the corresponding values of  $c$  and  $h$ .

\* *Biometrika*, xvii, pp. 364-387 (1925).

It is apparent that the penultimate form effectively bridges the great gap between samples of 1000 or less and the ultimate

TABLE A.

$h$	$m$	$n$	Mean - Mode		Standard Deviation		$\beta_1$		$\beta_2$	
			Ultimate	Pen-ultimate	Ultimate	Pen-ultimate	Ultimate	Pen-ultimate	Ultimate	Pen-ultimate
0	$\infty$	$\infty$	0	0	0	0	1.2986	1.2986	5.400	5.400
0.02	6.8493	$264 \cdot 10^8$	.0825	.0769	.1833	.1787	1.2986	1.0503	5.400	4.878
0.04	4.6699	$637 \cdot 10^8$	.1182	.1020	.2626	.2499	1.2986	.8436	5.400	4.451
0.06	3.6528	7228	.1470	.1169	.3266	.3039	1.2986	.6709	5.400	4.100
0.08	3.0000	677	.1732	.1261	.3848	.3504	1.2986	.5267	5.400	3.810

TABLE B.

$h$	$n$	$m$	Mean - Mode		Standard Deviation		$\beta_1$		$\beta_2$	
			Pen-ultimate	Actual	Pen-ultimate	Actual	Pen-ultimate	Actual	Pen-ultimate	Actual
.0768	1000	3.0902	.1249	.1262	.3433	.3514	.548	.618	3.852	4.088
.0845	500	2.8782	.1276	.1287	.3604	.3704	.498	.570	3.751	4.003
.0967	200	2.5758	.1309	.1314	.3874	.4009	.425	.495	3.607	3.875
.1073	100	2.3263	.1334	.1323	.4124	.4294	.368	.429	3.493	3.765
.1154	60	2.1281	.1355	.1318	.4340	.4545	.328	.376	3.414	3.677

form for very large samples. The distance from mode to mean is given correctly for samples somewhere between 100 and 200 and

is underestimated by an amount which seems to attain a maximum of about 1% for samples of about 1000, whereas the value for the ultimate form is over 7% in error for samples of nearly a billion. The standard deviation is given by the penultimate form with a negative error of about  $2\frac{1}{2}\%$  at 1000 and only about  $4\frac{1}{2}\%$  at 60, while the ultimate form is nearly 10% out at 1000, and just under 3% at a billion. In both comparisons the largest deviations occur in the  $\beta$  coefficients. The latter are consistently too low in the penultimate form for samples of 1000 and less, and probably do not attain a close approximation until the sample number is nearly a million, while an equally good approximation to the ultimate values  $\beta_1 = 1.299$  and  $\beta_2 = 5.4$  would only be attained by such incredibly large samples as are represented by values of about .004 for  $h$  (c.  $10^{66}$ ). The changes in  $\beta_1$  and  $\beta_2$  with varying  $h$ , together with the actual values for samples up to 1000, are shown in Figs. 2 and 3.

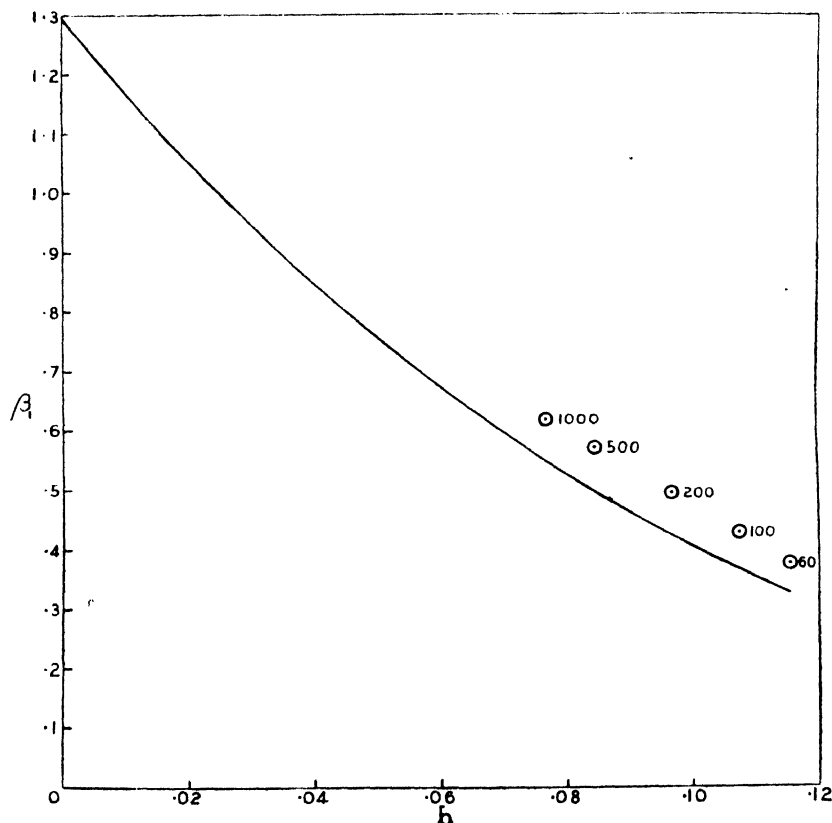


Fig. 2. Change in  $\beta_1$  with sample size as indicated by the penultimate formula, with actual values for samples up to 1000.

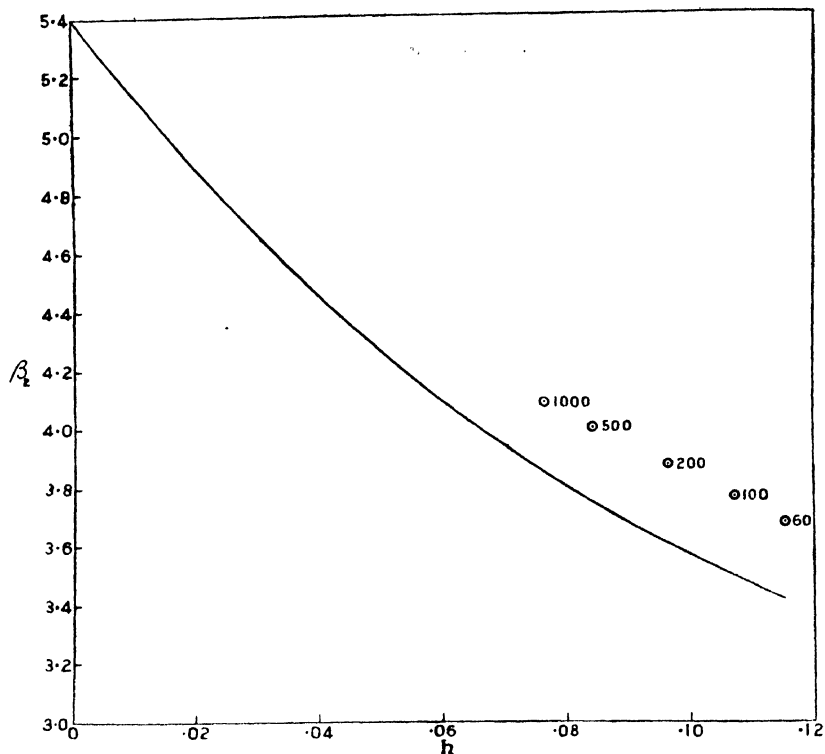


Fig. 3. Change in  $\beta_2$  with sample size as indicated by the penultimate formula, with actual values for samples up to 1000.

### 6. Summary.

The limiting distribution, when  $n$  is large, of the greatest or least of a sample of  $n$ , must satisfy a functional equation which limits its form to one of two main types. Of these one has, apart from size and position, a single parameter  $h$ , while the other is the limit to which it tends when  $h$  tends to zero.

The appropriate limiting distribution in any case may be found from the manner in which the probability of exceeding any value  $x$  tends to zero as  $x$  is increased. For the normal distribution the limiting distribution has  $h = 0$ .

From the normal distribution the limiting distribution is approached with extreme slowness; the final series of forms passed through as the ultimate form is approached may be represented by the series of limiting distributions in which  $h$  tends to zero in a definite manner as  $n$  increases to infinity.

Numerical values are given for the comparison of the actual with the penultimate distributions for samples of 60 to 1000, and of the penultimate with the ultimate distributions for larger samples.

# ON ERRORS IN THE MULTIPLE CORRELATION COEFFICIENT DUE TO RANDOM SAMPLING

BY

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The use of the multiple correlation coefficient in weather forecasting was suggested by Sir Gilbert Walker in 1910.<sup>1</sup> The application of the method consisted in regarding the monsoon rainfall of India (*e.g.*) as a dependent variate, affected by a number ( $n$ ) of independently varying factors, such as snowfall accumulation, pressure in Mauritius, rainfall at Zanzibar, etc. A sample for each of these factors is available for the number of years for which data have been recorded. This was about thirty in 1910, but with the passage of time a considerably larger sample can now be collected. The expression for the multiple correlation  $R_{1 \cdot 234 \dots (n+1)}$  is then given by

$$1 - R^2_{1 \cdot 23 \dots (n+1)} = (1 - r^2_{12})(1 - \rho^2_{13 \cdot 2})(1 - \rho^2_{14 \cdot 23}) \dots (1 - \rho^2_{1(n+1) \cdot 23 \dots n}),$$

where the dependent factor is represented by 1 and the others in succession by the numbers 2, 3, 4 . . . ( $n+1$ ).  $r_{12}$  is the correlation between factors 1 and 2, while  $\rho_{1s \cdot 23 \dots (s-1)}$  is the partial correlation between factors 1 and  $s$ , regarding the factors 2, 3, . . . ( $s-1$ ) as constant.  $R$  is then taken to be the positive square root of the value reached for  $R^2$ . It is important for the meteorologist to be able to determine the significance of  $R$ , once he has obtained a value for it. It has generally been assumed<sup>2</sup> in the past that the variance (or the square of the standard deviation) of  $R$ ,

in a large number of samples, is  $\frac{(1 - R^2)^2}{N}$ , where  $N$  is the size of the

sample and  $R$  is the multiple correlation in the population from which the sample is taken. This is not, of course, known, and the value of  $R$  reached for the particular sample is substituted. Doubt has been felt, however, in many minds as to the complete applicability of this formula. The theory is not altogether analogous to that of the simple correlation coefficient  $r$ . For instance, it has long been recognised that, even if the multiple correlation is zero in the population, positive values of varying size will occur in samples, owing to the errors of random sampling. Hence the mean value of  $R$ , deduced from a large number of samples, is a positive quantity, and not equal to the correlation in the population as a whole. Naturally, this positive bias of  $R$  has to be taken account of in estimating the significance of any sample value. If we assume that the

formula  $\frac{(1 - R^2)^2}{N}$  holds generally, what value of  $R$  should we insert when there is, in fact, no real relationship? What is the theoretical mean value

<sup>1</sup> "Correlation in Seasonal Variation of Weather," ii., *Calcutta, Ind. Met. Mem.*, xxi., Part 2, 1910.

<sup>2</sup> See Sir Gilbert Walker, *loc. cit.*, p. 25.

of  $R$  in a large number of samples taken from a normal population with no real correlation? Yule observed generally that this mean value will vary directly as the number of independent variables and inversely as the number of observations in the sample.<sup>3</sup> But the first attempt to give a more exact answer to these questions was made by Isserlis,<sup>4</sup> who dealt for simplicity with the case of three variables, *i.e.*  $n=2$ . He showed that the principal term in the excess of the mean value of  $R$  over the true value in the population is  $\frac{(1-R^2)^2}{2NR}$ , and that, correct to  $\frac{1}{N}$ , the formula

$\sigma^2_R = \frac{(1-R^2)^2}{N}$  remains true when allowance is made for this displacement

in the position of the mean. Unfortunately, the approximation for mean  $R$  is not sufficient, for it may give values greater than unity, and becomes infinite for  $R=0$ . The size of the sample is, of course, a material factor. But while the theory is based on the assumption of large samples, this only means that terms of the order of  $\frac{1}{N^2}$  can be neglected in comparison

with those of order  $\frac{1}{N}$ . A statistically large sample is one of 50 and upwards, and, although experience has shown in the past that a different theory altogether is required for small samples, such theory usually becomes almost indistinguishable from the large sample theory for samples of 40 to 50.

Altogether, the importance of the problem to meteorologists, and the inadequacy of such theoretical results as have hitherto been given, seemed to the author to justify his approaching the problem experimentally. The problem considered was: an infinite population, following the normal law, is given. Four independent random samples, first of 30 and then of 40, are drawn from this population. These are supposed to represent the distributions of four variates, the dependent variate  $u$ , and three independent variates,  $x$ ,  $y$  and  $z$ . Owing to the conditions of the experiment, there can be no real multiple correlation of  $u$  with a linear function of  $x$ ,  $y$ ,  $z$ . But in the sample a value is obtained which can be calculated. The experiment is then repeated a sufficient number of times to enable a fairly accurate value of (*a*) the mean  $R$ , and (*b*) the variance of  $R$ , to be determined. Such an experiment, if successful, can, of course, only be a first stage in the determination of the distribution of  $R$ , as only the particular case of no correlation in the population is considered. It is hoped in a further study to introduce a real known correlation between  $u$  and  $x$ ,  $y$ ,  $z$ , and then to study the distribution of  $R$  under these conditions. But the present investigation will give the meteorologist some information about the order of the correlations he may expect to get by random sampling, when the selected factors have really no influence whatever on the phenomenon whose variation he is considering. Then by a process of elimination of such a fictitious relationship, he is able to gain an appreciation of the measure of real dependence left, if there is any.

#### DETAILS OF THE EXPERIMENT.

A population was selected having twenty-five classes, represented by the numbers 0,  $\pm 1$ ,  $\pm 2$ , . . . .  $\pm 12$ . Numbers were assigned to these

<sup>3</sup> *Introduction to the Theory of Statistics*, p. 249 (Griffin), and *Proc. Roy. Soc., A*, lxxix., 1907, p. 193.

<sup>4</sup> *Phil. Mag.*, **34**, 1917, p. 205.

classes which were taken from a table of the normal probability integral. The distribution is therefore symmetrical about the class 0, and the frequencies are as in the following table:

TABLE I.—FREQUENCIES.

0	±1	±2	±3	±4	±5	±6	±7	±8	±9	±10	±11	±12
298	285	249	199	146	98	60	33	17	8	3	1	1

Total population = 2498.

Mean = 0. Standard deviation ( $\sigma$ ) = 3.34.

The particular method of sampling adopted was to use Tippett's series of random numbers. This consists of a series of four figure numbers, ranging from 0000 to 9999 in a perfectly random order. The numbers are assigned to the particular classes into which the population is divided in quantities proportional to the frequencies. In the present case, since there are 10,000 numbers and just under 2500 in the population, it is possible to run through the list four times before exhausting it. The table is gone over in any systematic manner, *i.e.* by following columns or rows: where a number is reached, the class corresponding to that number is noted. This is done until we have a sample of 40 numbers for  $n$ . The same is done for  $x$ ,  $y$  and  $z$ , and this then completes one set of data for the determination of  $R$ . Actually forty such sets were taken and worked out. There are many advantages in using this particular method of sampling. It fulfils all the conditions of sampling from an infinite population. The crude method of drawing numbers from a hat, or balls from a bowl, requires the slips, or balls, to be replaced after each draw. Furthermore such methods have in the past been proved to be unsatisfactory, for slips of paper tend to stick together, and balls in an urn, owing to their variation in size, weight, etc., are not usually randomly distributed, even with a great deal of mixing. The random nature of Tippett's numbers has been well established—the present experiment is one more illustration of this—and sampling conducted by means of them is as satisfactory as it can be. The sheets have now been issued as one of the publications of the Biometric Laboratory, University College, London,<sup>5</sup> and the author is indebted to Professor Karl Pearson for permission to use the manuscript sheets in his possession.

The case of four variates was chosen for purposes of illustration, the values of  $R$  being calculated as follows: Let  $\Delta$  denote the determinant  $|r_{ab}|$ , where  $a$  and  $b$  run from 1 to  $n+1$ , and  $r_{ba} = r_{ab}$ , while  $r_{aa} = 1$ . Let  $\Delta_{,,}$  denote the determinant formed from  $\Delta$  by deleting the first row and column. Then <sup>6</sup>  $1 - R^2 = \frac{\Delta_{,,}}{\Delta}$ , and we have for the special case of  $n = 3$ ,

$$\Delta_{,,} = 1 - r_{23}^2 - r_{24}^2 - r_{34}^2 + 2r_{23}r_{24}r_{34}.$$

$$\Delta = \Delta_{,,} + (r_{14}r_{23} - r_{12}r_{34})^2 - r_{13}^2r_{24}^2 \{ 2(r_{14}r_{23} + r_{12}r_{34}) - r_{13}r_{24} \} \\ - r_{12}^2 - r_{13}^2 - r_{14}^2 + 2(r_{12}r_{13}r_{23} + r_{12}r_{14}r_{24} + r_{13}r_{14}r_{34}).$$

**RESULTS.**—The two sets of forty values obtained for  $R$  are set out in the tables below, and the mean value and the standard deviation (square

<sup>5</sup> *Tracts for Computers*, xv., 1927. Cambridge University Press.

<sup>6</sup> K. Pearson, *Biometrika*, viii., 1912, p. 439. Also *Phil. Trans. R. Soc., A*, 200, 1901, p. 17.

root of variance) are worked out. Probable errors are attached, these being based on the assumption that the two constants are normally distributed with variance respectively  $\frac{\sigma^2}{N}$  and  $\frac{\sigma^2}{2N}$ , where  $N$  is the size of the sample (40 in this case), while we insert as the best estimate of  $\sigma$  the value obtained from the distribution itself. Results are given for both  $R$  and  $R^2$ , as some workers prefer to work with one, and some with the other. The theory of the distribution of  $R^2$ , too, is in some respects simpler than that of  $R$ .

TABLE II.—DISTRIBUTION OF  $R$  (30 YEARS' DATA).

Value of $R$	.08	.09	.10	.11	.15	.17	.18	.21	.22	.23	.25	.27	.30	.31	.32	.33	.34	.35	.36	.37	.40	.43	.45	.48	.51	.54
Frequency	1	1	2	1	1	1	1	3	2	2	2	2	2	2	3	2	1	1	2	3	1	1	1	1	1	1

Mean value of  $R = .288 \pm .012$ , of  $R^2 = .096 \pm .007$ .

Standard deviation of  $R = .113 \pm .009$ , of  $R^2 = .068 \pm .005$ .

It is recognised that complete information with regard to the distribution of  $R$  can hardly be gained from a sample of 40 values. The labour involved, however, in calculating even this moderate number was fairly strenuous, involving, as it did, the computation of 240 coefficients of correlation and 160 means and standard deviations. Enough has been done to indicate the kind of values, with their frequency, that are likely to arise by chance. For example, two values, or 5 per cent, occurred over .5, and a similar percentage below .1. The author has confidence that the mean value and standard deviation are very close to their real values. Divided into four blocks of ten, these gave .276, .280, .303 and .293 for the mean value, and the highest and lowest of these do not differ from the value .288 by more than the probable error. In a later section these results will be compared with theory.

TABLE III.—DISTRIBUTION OF  $R$  (40 YEARS' DATA).

Value of $R$	.01	.06	.09	.10	.15	.16	.17	.18	.19	.20	.21	.22	.25	.26	.28	.29	.31	.32	.33	.34	.40	.42	.45	.48	.52
Frequency	1	1	1	1	2	2	1	2	2	3	3	1	2	1	1	2	2	2	2	3	1	1	1	1	1

Mean value of  $R = .252 \pm .012$ , of  $R^2 = .075 \pm .007$ .

Standard deviation of  $R = .111 \pm .008$ , of  $R^2 = .062 \pm .005$ .

Again these values are believed to be representative, as is evidenced by the values, for the mean, of .239, .241, .271 and .256 for four blocks of ten. Only one value greater than .5 occurred, while one (the chance for which must be exceedingly small), was found to be as low as .01.

Although the distribution of  $R$  is far from normal, it is interesting to see that, in Table II., 22 values of  $R$  fall within, and 18 without, the limits  $.288 \pm (.6745)(.113)$ , while in Table III., 21 values fall within, and 19 without, the limits  $.252 \pm (.6745)(.111)$ . It should be noted that the most frequently occurring value is not the mean, but some value lower than this (about .2), and the distribution is obviously skew, rising fairly rapidly for low values of  $R$ , and only tailing off gradually after .2 has been passed. A suggestion of a second mode, at about .35, is obviously due

to the sample we have taken being inadequately representative of the distribution as a whole, owing to its size.

#### PROOF OF THE RANDOM NATURE OF THE SAMPLES.

A good test of the randomness of the forty samples, and incidentally of the method of sampling adopted, is afforded by examining the three distributions (i.) of the 160 means, (ii.) of the 160 standard deviations, and (iii.) of the 240 coefficients of correlation. The relevant facts as to these are set out below (for 40 years' data).

(i.) Range from  $-1.175$  to  $+1.3$ . Mean  $-.07$  (true mean 0). Standard deviation  $= \frac{3.34}{\sqrt{40}} = .53$ . Probable error  $\pm .36$ . 86 values lie within, and 74 without, the limits  $0 \pm .36$ . Divergence of actual from true mean is only one-seventh of standard deviation.

(ii.) Range from  $2.54$  to  $4.24$ . Mean  $3.26$  (true value  $3.34$ ). Standard deviation  $\frac{3.34}{\sqrt{80}} = .37$ . Probable error  $\pm .25$ . 82 values lie within, and 78 without, the limits  $3.34 \pm .25$ . Divergence from true value is only one-fifth of standard deviation.

(iii.) Range from  $-.48$  to  $+.41$ . Mean  $+.002$  (true value 0). Standard deviation  $\frac{1}{\sqrt{40}} = .16$ . Probable error  $\pm .11$ . 121 values lie within, and 119 without, the limits  $0 \pm .11$ . Divergence from true value is exceedingly small.

#### COMPARISON WITH THEORY.

R. A. Fisher<sup>7</sup> has given, for the particular case of no real correlation, the distribution in random samples of  $N$  of a multiple correlation  $R$ , obtained by correlating  $n$  independent variates with a dependent variate. His result is that the frequency of occurrence within the elementary range between  $R^2$  and  $R^2 + dR^2$  of any specified value is given by

$$\frac{\Gamma\left(\frac{N-1}{2}\right)}{\Gamma\left(\frac{N-n-1}{2}\right) \Gamma\left(\frac{n}{2}\right)} (R^2)^{\frac{n-2}{2}} (1-R^2)^{\frac{N-n-3}{2}} dR^2.$$

It follows that the probability of a value less than any specified amount  $R$  is given by the *Incomplete Beta Function*<sup>8</sup>

$$I_{R^2}\left(\frac{n}{2}, \frac{N-n-1}{2}\right).$$

A table of this function is in course of preparation by the author. Such a table will have many applications: among others it will enable the meteorologist to test directly the significance of any value of  $R$  he obtains, and will indicate to him whether it has arisen by chance, or whether there is a real relationship between the factors tested. To take one example, suppose  $n=3$ ,  $N=40$ . The probability that a value of  $R$  greater than .5

<sup>7</sup> "Influence of Rainfall on the Yield of Wheat at Rothamsted," *Phil. Trans. R. Soc., B*, **213**, p. 91, 1924.

<sup>8</sup>  $I_x(p, q)$  is defined as  $\int_0^x x^{p-1}(1-x)^{q-1}dx \div \int_0^1 x^{p-1}(1-x)^{q-1}dx$ .

will occur is  $1 - I_{.25}(1.5, 18)$ . A simple calculation gives this as  $\cdot 0148$ , so that a value of  $R$  greater than  $\cdot 5$  may be expected to occur by chance in a population with no real relationship about once in sixty-eight times, when there are four variates and the data extend over 40 years. Actually in our experiment we reached such a value only once out of forty. If such a value occurred in practice, it would be regarded as showing a real relationship, for we have to make up our minds where to draw the line between what is significant and what is not, and R. A. Fisher<sup>9</sup> has suggested a probability of  $\cdot 05$ , or one in twenty, as a suitable point.

To return to the actual distribution, we have the following values for the statistical constants associated with it:

$$\text{Mean value of } R = \frac{\Gamma\left(\frac{n+1}{2}\right)}{\Gamma\left(\frac{n}{2}\right)} \cdot \frac{\Gamma\left(\frac{N-1}{2}\right)}{\Gamma\left(\frac{N}{2}\right)}.$$

This expression, which is difficult to calculate as it stands, may be expressed approximately as

$$\frac{\left(\frac{n-1}{2}\right)!}{\left(\frac{n-2}{2}\right)!} \sqrt{\frac{2}{N-\frac{3}{2}}}.$$

This approximation depends on  $N$  being fairly large, but it can also be applied without great loss of accuracy to  $n$  as well as to  $N$ , and we have the convenient formula

$$\text{Mean } R \doteq \sqrt{\frac{2n-1}{2N-3}}.$$

Also (standard deviation)<sup>2</sup> of  $R = \frac{n}{N-1} - (\text{mean } R)^2$ ,

$$\doteq \frac{N-n-1}{(N-1)(2N-3)}.$$

The corresponding formulæ for  $R^2$  are:

$$\text{Mean value of } R^2 = \frac{n}{N-1},$$

$$(\text{standard deviation})^2 \text{ of } R^2 = \frac{2n(N-n-1)}{(N-1)(N^2-1)}.$$

Let us compare these results with experiment.

### 30 YEARS' DATA.

$$N=30, n=3.$$

*Theory*: mean  $R = \cdot 299 \pm \cdot 012$ .      *Experiment*: mean  $R = \cdot 288$ .  
 $\sigma_R = \cdot 119 \pm \cdot 009$ .       $\sigma_R = \cdot 113$ .

Alternatively for  $R^2$  we have the following:

*Theory*: mean  $R^2 = \cdot 103 \pm \cdot 007$ .      *Experiment*: mean  $R^2 = \cdot 096$ .  
 $\sigma_{R^2} = \cdot 077 \pm \cdot 005$ .       $\sigma_{R^2} = \cdot 068$ .

<sup>9</sup> *Statistical Methods for Research Workers*, p. 79 et seq. Oliver & Boyd, 1925.  
 See also *Phil. Trans. R. Soc.*, B, **213**, p. 95, 1924.

We may take it that the theoretical results are borne out very well by the results of our experiment. In only one case, that of  $\sigma_{R^2}$ , is the divergence greater than the probable error of the constant.

## 40 YEARS' DATA.

$$N = 40, n = 3.$$

<i>Theory</i> : mean $R = .257 \pm .012$ .	<i>Experiment</i> : mean $R = .252$ .
$\sigma_R = .104 \pm .008$ .	$\sigma_R = .111$ .
mean $R^2 = .077 \pm .007$ .	mean $R^2 = .075$ .
$\sigma_{R^2} = .059 \pm .005$ .	$\sigma_{R^2} = .062$ .

Here again the agreement is excellent.

The actual theoretical distribution is shown in Fig. 1, fitted to the observed values. The size of the sample (40) is, of course, too small

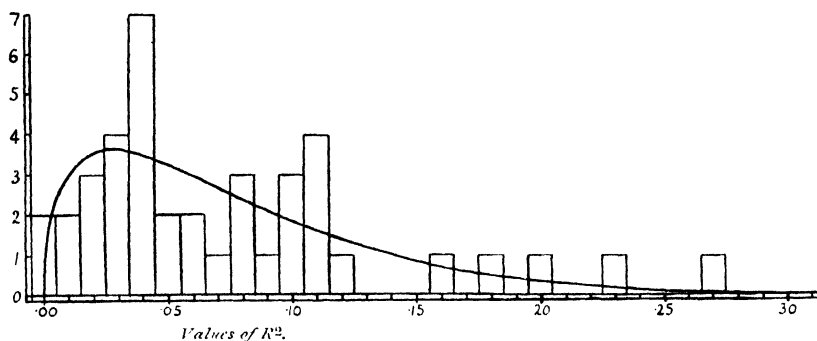


FIG. 1.

for a regular gradation of values to be obtained. The theoretical frequencies to be expected are given in Table IV.; these were obtained by using a planimeter on a large-scale drawing of the curve.

TABLE IV.—DISTRIBUTION OF  $R^2$  (40 YEARS' DATA).

Value of $R^2$ .	.00	.01	.02	.03	.04	.05	.06	.07	.08	.09	.10	.11	.12	.13	.14	.15	.16	.17	.18	.19	.20	above .20
Actual frequency	2	2	3	4	7	2	2	1	3	1	3	4	1	0	0	0	1	0	1	0	1	2
Theoretical	1.5	2.9	3.5	3.5	3.5	3.2	2.9	2.6	2.3	2.1	1.8	1.6	1.4	1.2	1.0	0.9	0.7	0.6	0.5	0.4	0.3	1.6

An application of the "goodness of fit" test<sup>10</sup> gives  $\chi^2 = 17.56$ ,  $n' = 22$  (clubbing together the frequencies above .2). Thus  $P = .68$ , and we conclude that in 68 out of 100 trials we should get in random sampling a fit as bad or worse than that observed (assuming the distribution to be normal).

Thus a good measure of agreement between theory and experiment is indicated.

## CASE WHERE REAL CORRELATION IS PRESENT.

We have already indicated that a measure of the real relationship between a group of factors may be gauged by eliminating such correlations

<sup>10</sup> See Pearson, K., *Tables for Statisticians, Introduction*, p. xxxi, and Table xii., pp. 26-28. Cambridge University Press, 1914.

as might arise by chance on the basis of the foregoing theory, and estimating the significance of what is left. The actual theory, where correlation is present in the sample as a whole, is more difficult, and has not yet been fully worked out. It is as well to point out that, if the meteorologist chooses those factors which seem to have an upward or downward trend along with the factor he seeks to predict, he is really selecting out of a large number of factors those which give the maximum relationship. This still further complicates the problem, and it is therefore advisable to repeat what R. A. Fisher<sup>11</sup> said in 1923, namely, that the factors to be used in estimating the correlation should be chosen without reference to the trend of the dependent variate (*e.g.* rainfall). It is, of course, generally recognised that there is a limit (the number in the sample) to the number of factors that can be employed, and if the desire is to increase this number, the calculation should only be performed when a large sample is available. There are considerable arithmetical difficulties, however, in the way of extending the number of factors beyond five or six, and so the number of years of data (40), at present available for meteorological phenomena, may be regarded as quite adequate for the purpose in hand.

It is fairly certain that the formulæ adduced in this paper for the excess of the mean value of  $R$ , as determined from a number of samples, over the value in the population as a whole, give the maximum values. As  $R$  increases from zero, this excess will decrease steadily till it vanishes altogether at  $R=1$ . A similar argument applies to the case of the variance.<sup>12</sup>

### CONCLUSIONS.

The experiment has shown, and theory has confirmed, that when there is no real multiple correlation between the factors studied, definite values for the mean of a large number of samples, and for the variance, occur. These are given by the formulæ cited, and there is no question that the formula  $\frac{(1-R^2)^2}{N}$ , whatever its applicability may be where correlation is present, fails entirely when  $R=0$ . It does not matter whether we apply this formula with  $R=0$  or  $R$  equal to the value derived from a particular sample. In both cases the result reached for the standard deviation (between  $\cdot 15$  and  $\cdot 16$  for  $N=40$ ) is a good deal too large. It is further shown that R. A. Fisher's theory, applied to the limited sample of experiments, gives a reasonably good fit, as measured by Pearson's criterion. The probability that any specified value of  $R$  will be exceeded by chance is given by a particular mathematical function, tables of which are in course of preparation. The form of the results for the more general case of real relationship existing between the factors is suggested, but the theory is still far from complete.

<sup>11</sup> *Loc. cit.*, p. 95.

<sup>12</sup> P. Hall, in *Biometrika*, xix., 1927, p. 104, has obtained for the mean value of  $R^2$  the approximate result  $\frac{1-R^2}{N} - (n-2R^2)$ , correct to  $\frac{1}{N}$ . He also gives for the standard deviation of  $R^2$  the value  $\frac{2R(1-R^2)}{\sqrt{N}}$ , approximately, a result which is, however, insufficient, as  $R \rightarrow 0$ .

## ADDENDUM.

In the meantime the function  $I_{R^2}\left(\frac{n}{2}, \frac{N-n-1}{2}\right)$  may be expressed in terms of (a) a binomial series, or (b) a symmetrical integral which is tabulated, according as  $n$  is even or odd. Thus for  $n$  even the probability that  $R$ , obtained from a random sample of  $N$  observations, should exceed any specified value, is

$$P = (1+X)^{-\frac{1}{2}(N-3)} \left\{ 1 + \frac{N-3}{2} X + \frac{(N-3)(N-5)}{2 \cdot 4} X^2 + \dots + \frac{(N-3) \dots (N-n+1)}{2 \cdot 4 \dots (n-2)} X^{\frac{n}{2}-1} \right\}, \text{ where } X = \frac{R^2}{1-R^2}.$$

For  $n$  odd, the corresponding result is

$$P = I_{1-R^2}\left(\frac{N-2}{2}, \frac{1}{2}\right) + 2 \sqrt{\frac{X}{\pi}} \cdot \frac{\Gamma\left(\frac{N-1}{2}\right)}{\Gamma\left(\frac{N-2}{2}\right)} \cdot (1+X)^{-\frac{N-3}{2}} \times \left\{ 1 + \frac{N-4}{3} X + \frac{(N-4)(N-6)}{3 \cdot 5} X^2 + \dots + \frac{(N-4) \dots (N-n+1)}{3 \cdot 5 \dots n-2} X^{\frac{n-3}{2}} \right\}.$$

The first part of this result,  $I_{1-R^2}\left(\frac{N-2}{2}, \frac{1}{2}\right)$ , can be read off directly from "Student's" Table of  $t$  (in *Metron*, Vol. V., No. 3, 1925, p. 26), by taking  $t = \sqrt{(N-2)X}$ ,  $n = N-2$ , subtracting the tabled value from unity, and then doubling. Unfortunately this table only extends as far as  $n = 20$  and does not give many places. For higher values the following approximate formula<sup>13</sup> (correct to at least one per cent) should be used:

$$I_{1-R^2}\left(\frac{N-2}{2}, \frac{1}{2}\right) = \sqrt{\frac{2}{(N-4)\pi X}} \cdot (1+X)^{-\frac{1}{2}(N-3)} \cdot \left(1 - \frac{1+X}{NX+2(1-X)}\right).$$

In the second part  $\frac{\Gamma\left(\frac{N-1}{2}\right)}{\Gamma\left(\frac{N-2}{2}\right)}$  may be replaced by its approximate value

$\sqrt{\frac{2(N-5)}{4}}$ , and the binomial series involved is the same as that for  $n$  even.

The advantage of these formulæ over those given by Fisher<sup>14</sup> is that  $n$ , the number of independent variates, only enters into the  $P$ -expressions to limit the number of terms of the series to be taken. The parts outside, and the successive terms, can be calculated once and for all for a number of typical values of  $N$  and  $R$ . Then the required answer is got by including  $\frac{n}{2}$  or  $\frac{n-1}{2}$  terms of the series, according as  $n$  is even or odd.

The meteorologist can by this means see at once the effect on the probability that a particular value of  $R$  will be exceeded by chance, of increasing, one at a time, the number of independent variates employed.

<sup>13</sup> For this and other approximations to the *Incomplete Beta Function*, see *Biometrika*, xix., 1927, pp. 1-38, and also xvii., 1925, pp. 68-78, and 469-472.

<sup>14</sup> *Phil. Trans. R. Soc., B*, **213**, p. 93. Also *Metron*, V., No. 3, p. 16. In the *Metron* result, for  $\sqrt{\pi} \sqrt{\frac{n_2-2}{n}}$  in the denominator ( $n$ , odd), read  $\sqrt{\pi} \sqrt{\frac{n_2-2}{n}} \cdot \frac{n_2-2}{2}!$ .

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## Table of Significant Values of the Multiple Correlation Coefficient

The following table, computed from advance proofs of R. A. Fisher's new table of " $\alpha$ ," by kind permission of the author,\* gives the values of the multiple correlation coefficient for two distinct levels of significance, and is based on the theory outlined in *Memoirs*, Vol. 2, No. 13. Part I. (5 per cent. point) gives the values that would occur on the average once in twenty times, in random sampling from uncorrelated material. Part II. (1 per cent. point) is a severer test, giving as it does the values that would arise by chance once in a hundred times, where there was no real relation. As the distribution is not normal, the "probable error" ceases to have its customary significance and should be replaced by such a table as this one, based on the exact distribution. In general the

### PART I—5 PER CENT POINT.

Degrees of freedom $=N-n-1$	Number of Independent Variables $=n$ .				
	2	3	4	5	6
1	.90875	.990228	.990444	.990566	.990644
2	.9747	.9830	.9873	.9898	.99149
3	.9297	.9501	.9612	.9683	.9732
4	.8811	.9120	.9299	.9416	.9499
5	.8356	.8743	.8978	.9136	.9252
6	.7947	.8391	.8668	.8861	.9004
7	.7583	.8067	.8378	.8599	.8765
8	.7261	.7771	.8108	.8351	.8536
9	.6972	.7502	.7858	.8119	.8320
10	.6713	.7257	.7627	.7902	.8116
11	.6480	.7032	.7414	.7700	.7924
12	.6269	.6826	.7216	.7511	.7744
13	.6077	.6636	.7032	.7334	.7574
14	.5901	.6461	.6861	.7168	.7414
15	.5739	.6298	.6701	.7012	.7263
16	.5589	.6147	.6551	.6865	.7120
17	.5450	.6006	.6410	.6727	.6985
18	.5321	.5874	.6278	.6596	.6856
19	.5201	.5749	.6154	.6473	.6735
20	.5088	.5633	.6036	.6356	.6619
25	.4616	.5139	.5534	.5851	.6116
30	.4255	.4750	.5138	.5449	.5711
35	.3967	.4446	.4816	.5119	.5376
40	.3730	.4190	.4547	.4841	.5094
45	.3531	.3973	.4318	.4605	.4851
50	.3361	.3787	.4121	.4400	.4640
60	.3083	.3481	.3796	.4060	.4289
70	.2864	.3230	.3537	.3788	.4007
80	.2686	.3042	.3325	.3565	.3774
90	.2538	.2876	.3147	.3376	.3577
100	.2412	.2735	.2995	.3215	.3408

value reached for a multiple correlation coefficient should be at least equal to that given in the 5 per cent. point table before a significant result is claimed for the data. The table can be used in confidence even with samples of small size, where any criterion based on "probable error" would be impossible of application.

PART II.—1 PER CENT. POINT.

Degrees of freedom $=N-n-1$ .	Number of Independent Variates= $n$ .				
	2	3	4	5	6
1	.9999500	.9999691	.9999778	.9999826	.9999858
2	.99499	.99666	.99749	.99799	.99833
3	.9765	.9834	.9872	.9895	.99116
4	.9487	.9623	.9701	.9752	.9788
5	.9173	.9373	.9493	.9573	.9631
6	.8857	.9112	.9269	.9377	.9457
7	.8554	.8852	.9042	.9176	.9276
8	.8269	.8603	.8820	.8976	.9094
9	.8004	.8365	.8606	.8780	.8914
10	.7758	.8141	.8401	.8591	.8739
11	.7531	.7931	.8206	.8410	.8570
12	.7320	.7734	.8021	.8237	.8407
13	.7125	.7549	.7846	.8072	.8251
14	.6943	.7375	.7681	.7915	.8101
15	.6774	.7211	.7524	.7765	.7958
16	.6616	.7057	.7376	.7622	.7821
17	.6468	.6912	.7235	.7487	.7691
18	.6329	.6775	.7102	.7357	.7565
19	.6198	.6646	.6975	.7234	.7445
20	.6075	.6523	.6854	.7116	.7330
25	.5551	.6005	.6320	.6598	.6822
30	.5142	.5575	.5906	.6175	.6403
35	.4810	.5231	.5550	.5823	.6050
40	.4535	.4943	.5262	.5524	.5749
45	.4302	.4698	.5009	.5266	.5488
50	.4102	.4486	.4789	.5041	.5260
60	.3772	.4135	.4423	.4665	.4876
70	.3511	.3855	.4130	.4362	.4565
80	.3298	.3626	.3889	.4112	.4307
90	.3119	.3433	.3685	.3899	.4088
100	.2966	.3267	.3510	.3717	.3899

The table should be entered in the column corresponding to the number of independent variates (the  $n$  of the *Memoir* cited), and on the line determined, not by the size of the sample, but by the number of degrees of freedom in which the observations may deviate from the regression function. Thus if  $N$  is the number in the sample we enter on the line  $N-n-1$ . This test of significance for  $R$  is, in fact, the test whether the mean square ascribable to the regression function is or is not significantly greater than the mean square of deviations from the regression function.

ERRATUM.—In *Memoirs*, Vol. 2, No. 13, p. 36 (footnote), for "mean value" read "mean excess."

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\* *Statistical Methods for Research Workers*. Second Edition. Oliver and Boyd, 1928.



**The Correlation between Product Moments of any Order in Samples from a Normal Population.** By John Wishart, M.A., D.Sc., Statistical Department, Rothamsted Experimental Station. *Communicated by Professor E. T. WHITTAKER, F.R.S.*

(MS. received November 21, 1928. Read January 7, 1929.)

1. A PROBLEM of considerable importance in the theory of statistics is the determination of the accuracy with which a given sample of observations determines the characteristics of the population from which it is derived. Any parameter of this distribution can only in practice be estimated from the sample, and the degree of latitude in our choice of the right function enables considerations of suitability and efficiency of the particular moment statistic to be taken into account. As well as this, however, the probable errors of such frequency constants, or, to be more complete, the distribution of the constants in all possible samples, must be determined. These problems were formulated many years ago by Karl Pearson (1), and his important researches have stimulated and suggested much subsequent work. His probable error formulæ, however, are only applicable with strictness to large samples, *i.e.* they are approximations in  $N$ , the number of the sample, in the sense that terms of order  $\frac{1}{N}$  have been neglected in comparison with 1. Recent work has been directed to removing this limitation, and since the pioneer work of "Student" in 1908 (2) a great deal of progress has been made with the theory of the small sample. The experimenter need no longer be discouraged if he is unable to produce as many as fifty or sixty observations. It is still true, of course, that a small sample cannot be expected to give as accurate a determination of the population parameters as a large one, but the limits of error are now precisely defined in a way that was not possible before.

Certain simple results of the large sample theory on the correlation between the estimated direct and product moment coefficients in samples from a bi-variate population have prompted me in this paper to consider how far they are true exactly. The problems are of interest in themselves, but also for the tests they furnish of the normality or otherwise of any given body of data. Suppose  $x_{p1}, x_{p2}, x_{p3} \dots x_{pN}$  are the observed  $N$  values of a variate  $x_p$ , and  $x_{q1}, x_{q2}, \dots x_{qN}$  are the corresponding values

## 2 The Correlation between Product Moments of any Order

of a variate  $x_q$ , in a sample chosen from an infinite population having two correlated variables, both normally distributed. Let the estimate of the second order product moment coefficient (co-variance), obtained from the sample, be defined by

$$a_{pq} = \frac{1}{N} \sum_1^N (x_p - \bar{x}_p)(x_q - \bar{x}_q),$$

where  $\bar{x}_p$ ,  $\bar{x}_q$  are the mean values in the sample of the variates  $x_p$ ,  $x_q$  respectively. Then I have shown (3) that the correlation between the estimates of any two second order product moments, over all samples of  $N$  that can be drawn from the population, is given by

$$r_{a_{12} \cdot a_{34}} = \frac{\rho_{13}\rho_{24} + \rho_{14}\rho_{23}}{\{(1 + \rho_{12}^2)(1 + \rho_{34}^2)\}^{\frac{1}{2}}} \quad (1),$$

where  $\rho_{pq}$  is the correlation in the population between the  $p^{\text{th}}$  and  $q^{\text{th}}$  variates.

In particular, if we put 1=2 and 3=4 we have the correlation between the estimates  $a_{11}$  and  $a_{22}$  of the variances in the form

$$r_{a_{11} \cdot a_{22}} = \rho_{12}^2 \quad (2)$$

We may now go on to consider the problem of the correlation between estimated product moment coefficients of higher order than the second.

Let

$$m_{tu} = \frac{1}{N} \sum_1^N (x - \bar{x})^t (y - \bar{y})^u$$

be the estimate from the sample of the general product moment coefficient of the  $(t+u)^{\text{th}}$  order. No exact solution has hitherto been reached for the correlation between  $m_{tu}$  and  $m_{vw}$ , even in samples from a normal population. It depends, evidently, upon considering the bi-variate distribution, in all possible samples, of the estimates  $m_{tu}$  and  $m_{vw}$ , and determining from this distribution (a) the variances of  $m_{tu}$  and  $m_{vw}$  and (b) the product moment coefficient of  $m_{tu}$  and  $m_{vw}$ . This distribution is not known. R. A. Fisher (4) has recently shown, however, that if in place of the moment function estimates  $m_{tu}$  we calculate certain symmetric functions of the observations  $k_{tu}$  (defined below), which are analogous to the semi-invariants  $\kappa_{tu}$  of the sampled population, then the semi-invariants of the simultaneous distribution in samples of any number of the quantities  $k_{tu}$  can be determined from the solution of a problem in combinatorial analysis. It is therefore possible to generalise (1) in semi-invariant form, and it then appears that correlations, for samples from a normal population, can only exist between  $k_{tu}$  and  $k_{vw}$  when  $t+u=v+w$ , i.e. correlation can only exist between these symmetric functions as

defined when they are of the same order. It will be shown in this paper that the correlation between the estimates  $k_{t0}$  and  $k_{0t}$  is given by

$$r_{k_{t0}, k_{0t}} = \rho^t \quad (3),$$

which is therefore a generalisation of (2), and more generally that the correlation between  $k_{tu}$  and  $k_{vw}$ , subject only to the condition that  $v+w=t+u$ , is

$$r_{k_{tu}, k_{vw}} = \frac{\rho^{t-v}}{(t-v)!} \cdot \frac{F(-u, -v, t-v+1, \rho^2)}{\left\{ \frac{u!}{t!} F(-t, -u, 1, \rho^2) \cdot \frac{v!}{t!} F(-v, -w, 1, \rho^2) \right\}^{\frac{1}{2}}} \quad (4),$$

where F denotes the hypergeometric series

$$F(\alpha, \beta, \gamma, x) = 1 + \frac{\alpha \cdot \beta}{1! \gamma} x + \frac{\alpha(\alpha+1)\beta(\beta+1)}{2! \gamma(\gamma+1)} x^2 + \dots$$

and  $t-v$  is positive.

2. Consider an infinite population in which the variable can take the value  $x_v$  with probability  $P_v$  ( $v=0, 1, \dots, \infty$ ),  $P_v$  being positive quantities satisfying the relation  $\sum_{v=0}^{\infty} P_v = 1$ . Then the moment coefficients of this distribution, about zero as origin, are given by

$$\mu'_h = \sum_{v=0}^{\infty} x_v^h P_v,$$

or, about the mean  $\mu'_1$ , by

$$\mu_h = \sum_{v=0}^{\infty} (x_v - \mu'_1)^h \cdot P_v.$$

The semi-invariants  $\kappa_h$  are defined by the identical relation

$$\exp. \sum_{h=0}^{\infty} \frac{\kappa_h t^h}{h!} = \sum_{v=0}^{\infty} e^{x_v t} \cdot P_v.$$

They can therefore be expressed in terms of the moment coefficients in the following way:—

$$\kappa_h = h! \sum_{l=1}^h (-1)^{l-1} \sum_{(i)} \frac{(l-1)!}{i_2! i_3! \dots i_h!} \left( \frac{\mu_2}{2!} \right)^{i_2} \left( \frac{\mu_3}{3!} \right)^{i_3} \dots \left( \frac{\mu_h}{h!} \right)^{i_h} \quad h > 1,$$

the summation with respect to  $i$  being over all the non-negative integers  $i_2, i_3, \dots, i_h$  satisfying the relations  $i_2 + i_3 + \dots + i_h = l$ ,  $2i_2 + 3i_3 + \dots + hi_h = h$ .

Special cases are (5)

$$\left. \begin{aligned} \kappa_1 &= \mu'_1 \\ \kappa_2 &= \mu_2, & \kappa_3 &= \mu_3 \\ \kappa_4 &= \mu_4 - 3\mu_2^2 \\ \kappa_5 &= \mu_5 - 10\mu_3\mu_2 \\ \kappa_6 &= \mu_6 - 15\mu_4\mu_2 - 10\mu_3^2 + 30\mu_2^3 \end{aligned} \right\} \quad (5)$$

## 4 The Correlation between Product Moments of any Order

Alternatively the moment coefficients ( $\mu$ ) can be expressed in terms of the semi-invariants ( $\kappa$ ) by the formula

$$\mu_h = \sum_{l=1}^h \sum_{(i)} \frac{h!}{i_2! i_3! \dots i_h!} \left(\frac{\kappa_2}{2!}\right)^{i_2} \left(\frac{\kappa_3}{3!}\right)^{i_3} \dots \left(\frac{\kappa_h}{h!}\right)^{i_h} \quad h > 0.$$

For a bi-variate population the moment coefficients are conveniently designated by means of a double suffix notation. Thus about zero we have

$$\mu_{gh}' = \sum_{v=0}^{\infty} x_v^g y_v^h P_v,$$

and about the means  $\mu_{10}'$  and  $\mu_{01}'$  of the  $x$  and  $y$  variates respectively,

$$\mu_{gh} = \sum_{v=0}^{\infty} (x_v - \mu_{10}')^g (y_v - \mu_{01}')^h \cdot P_v.$$

The semi-invariants  $\kappa_{gh}$  are then defined by means of the identity

$$\exp. \sum_{g,h=0}^{\infty} \kappa_{gh} \cdot \frac{t_1^g t_2^h}{g! h!} = \sum_{v=0}^{\infty} e^{x_v t_1 + y_v t_2} \cdot P_v \quad . \quad . \quad . \quad (6)$$

The simplest of the relations between  $\kappa_{gh}$  and  $\mu_{gh}$  are the following:—

$$\left. \begin{aligned} \kappa_{11} &= \mu_{11} \\ \kappa_{21} &= \mu_{21} \\ \kappa_{31} &= \mu_{31} - 3\mu_{20}\mu_{11} \\ \kappa_{22} &= \mu_{22} - \mu_{20}\mu_{02} - 2\mu_{11}^2 \end{aligned} \right\} . \quad . \quad . \quad . \quad (7)$$

Obviously when either  $g$  or  $h$  is equal to 0 the relations are given by (5) on adding a 0 before or after the suffix there given.

From a sample of size  $N$  an estimate  $m_h$  is made of the moment coefficient  $\mu_h$  by calculating

$$m_h = \frac{1}{N} \sum_1^N (x - \bar{x})^h.$$

As an estimate of the semi-invariants  $\kappa$ , R. A. Fisher has determined a series of symmetric functions  $k$  in such a way that the mean value of any  $k$  over all samples shall be equal to the corresponding semi-invariant ( $\kappa$ ) in the sampled population. The simplest of his results are

$$\left. \begin{aligned} k_1 &= m_1' = \frac{1}{N} \sum_1^N (x) \\ k_2 &= \frac{N}{N-1} m_2 \\ k_3 &= \frac{N^2}{(N-1)(N-2)} m_3 \\ k_4 &= \frac{N^2}{(N-1)(N-2)(N-3)} \left\{ (N+1)m_4 - 3(N-1)m_2^2 \right\} \end{aligned} \right\} . \quad . \quad (5a)$$

Regarded as new variates, the quantities  $k_1, k_2, k_3 \dots$ , etc., will vary from sample to sample in a multi-variate distribution which can in general be specified by its moment coefficients, or, if we like, its semi-invariants. These can be determined in terms of the moment coefficients ( $\mu$ ), or semi-invariants ( $\kappa$ ) of the original population, either by straightforward, if laborious, algebra, or more directly and elegantly by means of combinatorial analysis. The original population must be infinite in extent, but may have any law of distribution. Thus, suppose we require to find the second semi-invariant, or the variance, of  $k_t$ .  $k_t$  is chosen as such a function of the observations in one sample that the mean value of  $k_t$  in all possible samples is  $\kappa_t$ . We then require the mean value of  $(k_t - \kappa_t)^2$ . This will be denoted by  $\kappa(t\ t)$ , and is a semi-invariant of the second order. The number  $2t$  is first divided in any chosen way into two parts of  $t$  units each. Now  $\kappa(t\ t)$  will consist of a number of terms comprising the  $\kappa$ 's of the original population, the first being that in  $\kappa_{2t}$  corresponding to the simple partition  $(t\ t)$  of the number  $2t$ . It will be associated with a coefficient  $\frac{1}{N}$ . The next term will involve  $\kappa_{2t-2}\kappa_2$  corresponding to the second order partition

$$\begin{pmatrix} t-1 & t-1 \\ 1 & 1 \end{pmatrix}$$

in which the two units to be taken together as partners in the second row can be chosen in  $t^2$  ways. There is, therefore, a numerical coefficient  $t^2$ , and an N-coefficient, according to Fisher's rules, of  $\frac{1}{(N-1)}$ . And so on. There are always two columns, for we are evaluating a second order semi-invariant, and each adds up to  $t$ , but the successive terms depend upon partitioning the number  $t$  into a varying number of parts, and there will thus be a varying number of rows, the sums of which specify the particular constituent semi-invariants involved. No row can add up to 1 since the formula involves semi-invariants about the mean of the population, and therefore cannot involve  $\kappa_1$ , while, according to Fisher's rules, no row of a two-column partition can contain a zero entry, because the N-coefficient would vanish in such a case. There will be a particular N-coefficient for every pattern, this depending only upon the number of rows, and not on the nature of the partition. The numerical coefficient is always the number of ways in which the second order partition can be set up. We shall assume in what follows that the original population is normally distributed. It follows that all  $\kappa$ 's higher than  $\kappa_2$  vanish, and the only partition left to consider is one of  $t$  rows, each containing one

## 6 The Correlation between Product Moments of any Order

unit in each column. This partition can be set up in  $t!$  different ways, regarding the two sets of  $t$  units each as aggregates of different objects, interchangeable within the column, but not between one column and another. The N-coefficient is

$$\sum_{r=1}^t \frac{(r-1)!}{r} \cdot \frac{\Delta^r 0^t}{N(N-1) \dots (N-r+1)},$$

where  $\Delta^r 0^t$  is defined as the  $r^{\text{th}}$  difference of  $|x|^t$  when  $x=0$ . The differences of the powers of zero are tabulated in a number of textbooks

(6). This N-coefficient is also  $\frac{1}{N}$ th of the leading term in the expression for  $k_t$  in terms of  $m_t$ , and lower order  $m$ 's. It should be emphasised here that the N-coefficient is the same in all the cases to be considered in this paper, since it depends only on the pattern, and not at all on the particular partition to be evaluated.

We may now write down directly the variance of  $k_t$ . It is, in fact,

$$t! \sum_{r=1}^t \frac{(r-1)!}{r} \cdot \frac{\Delta^r 0^t}{N(N-1) \dots (N-r+1)} \kappa_2^t, \quad \dots \quad (8)$$

in which  $\kappa_2$  may be replaced by  $\sigma^2$ , where  $\sigma$  is the standard deviation in the population.

For a bi-variate population in which the variates  $x$  and  $y$  are both normally distributed, we may conveniently use the notation  $k_{t_0}$  to express the  $t^{\text{th}}$  symmetric function of the observations of the  $x$ -variate, and  $k_{0t}$  to express the corresponding function of the  $y$ -variate.  $\kappa_{2_0}$  is then the second semi-invariant of the  $x$ -variate in the population, and may be equated to  $\sigma_1^2$ , while  $\kappa_{02}$  is the corresponding semi-invariant of the  $y$ -variate, and is equal to  $\sigma_2^2$ . The variances of  $k_{t_0}$  and  $k_{0t}$  are then both expressed by (8), if  $\kappa_2$  is replaced by  $\kappa_{2_0}$  and  $\kappa_{02}$  respectively, or by  $\sigma_1^2$  and  $\sigma_2^2$ .

Now to determine the correlation between  $k_{t_0}$  and  $k_{0t}$  in samples from our normal bi-variate population we require the product semi-invariant, or moment, of  $k_{t_0}$  and  $k_{0t}$ . The partition is the same as before, but the objects in the first column are to be regarded as of one kind, corresponding to the  $x$ -variate, while those of the second column are of another kind ( $y$ -variate). The number of ways in which the partition can be set up is still  $t!$  and the N-coefficient remains the same, but the addition along a row is to be expressed as  $\kappa_{11}$  (because the two 1's represent different objects). Now  $\kappa_{11}$  is the second order product semi-invariant of the  $x$  and  $y$  variates in the original population, and is equal to  $\mu_{11}$ , or to  $\sigma_1 \sigma_2 \rho$ , where  $\rho$  is the

correlation coefficient between the  $x$  and  $y$  variates in the population. The product semi-invariant of  $k_{t_0}$  and  $k_{0t}$  is therefore

$$t! \sum_{r=1}^t \frac{(r-1)!}{r} \cdot \frac{\Delta^r 0^t}{N(N-1) \dots (N-r+1)} \sigma_1^t \sigma_2^t \rho^t \dots \quad (9)$$

The correlation between  $k_{t_0}$  and  $k_{0t}$ , from (8) and (9), is then

$$r_{k_{t_0} \cdot k_{0t}} = \frac{\kappa \begin{pmatrix} t & 0 \\ 0 & t \end{pmatrix}}{\left\{ \kappa \begin{pmatrix} t & t \\ 0 & 0 \end{pmatrix} \cdot \kappa \begin{pmatrix} 0 & 0 \\ t & t \end{pmatrix} \right\}^{\frac{1}{2}}} = \rho^t \quad (10)$$

In the central expression the notation is designed so that the first row corresponds to the  $x$ -variate and the second to the  $y$ -variate, while the number of columns depends upon the order (in this case the second) of the semi-invariant to be evaluated.

Our first rule is then :

*The correlation between the  $t^{\text{th}}$  symmetric functions  $k_{t_0}$  and  $k_{0t}$  in samples from a bi-variate normal population is  $\rho^t$ , where  $\rho$  is the correlation coefficient in the sampled population.*

This is the required generalisation of equation (2). The condition that our pattern shall have no zero entries implies that for samples from a normal population no correlation can exist between  $k_{t_0}$  and  $k_{u_0}$  or between  $k_{t_0}$  and  $k_{0u}$ , unless  $u=t$ . This is an advantage over ordinary moments. For example, correlation does exist between the estimates  $m_2$  and  $m_4$  of the second and fourth moments obtained from samples of  $N$  drawn from a normal population. Its value is

$$r_{m_2 \cdot m_4} = \frac{\sqrt{3(N-1)}}{\sqrt{4N^2 - 9N + 6}}.$$

### 3. Correlation between Product Symmetric Functions of Two Variates.

$k_{tu}$  is defined as that symmetric function of the observations of the sample whose mean value, taken over all possible samples, is  $\kappa_{tu}$ , defined by (6). The simplest of these functions are

$$\left. \begin{aligned} k_{11} &= \frac{N}{N-1} m_{11}, & k_{21} &= \frac{N^2}{(N-1)(N-2)} m_{21} \\ k_{31} &= \frac{N^2}{(N-1)(N-2)(N-3)} \left\{ (N+1)m_{31} - 3(N-1)m_{20}m_{11} \right\} \\ k_{22} &= \frac{N^2}{(N-1)(N-2)(N-3)} \left\{ (N+1)m_{22} - (N-1)(m_{20}m_{02} + 2m_{11}^2) \right\} \end{aligned} \right\} \quad (11)$$

These correspond to the relations for  $\kappa_{tu}$  in terms of  $\mu$ 's given in (7), while

$$m_{tu} = \frac{1}{N} \sum_{i=1}^N (x - \bar{x})^t (y - \bar{y})^u.$$

## 8 The Correlation between Product Moments of any Order

The combinatorial problem for the case of product functions is analogous to that developed above for the direct functions. Thus, to determine the variance of  $k_{tu}$  we have to consider a two-column partition in each of which there are  $(t+u)$  units, but  $t$  are of one kind (and may be denoted by 1) while  $u$  are of another kind ( $\times$ ). The condition of normality assumed for the population, together with the rule that no zero can occur in a two-column partition, ensures that there shall be  $t+u$  rows each containing one unit, of the 1 or  $\times$  kind, in each column. The total number of ways in which the partition may be set up is  $(t+u)!$ , as in the simple case, but the partitions are divided up into sets which differ in their row totals.

Thus for the partition

1	1
1	1
1	1
.	.
.	.
.	.
.	.
$\times$	$\times$
$\times$	$\times$
.	.
.	.
.	.

in which there are  $t$  1's and  $u$   $\times$ 's in each column partnered by  $t$  1's and  $u$   $\times$ 's in the other column, the number of different ways in which the objects can be arranged is  $t!u!$ . This partition determines a term in  $\kappa_{20}^t \kappa_{02}^u$ , i.e. in  $\sigma_1^{2t} \sigma_2^{2u}$ , by adding along the rows, with numerical coefficient  $t!u!$  and N-coefficient as in (8), replacing  $t$  by  $t+u$ . If now at the junction of 1's and  $\times$ 's a pair in one column be interchanged we shall have a quartette

1	$\times$
$\times$	1

Each 1 can be chosen out of  $t$  to occupy this position in  $t$  ways, and likewise each  $\times$  can be chosen in  $u$  ways. In the remaining  $t-1$  rows all occupied by 1's the units can be arranged in  $(t-1)!$  ways, while in the remaining  $u-1$  rows occupied by  $\times$ 's there are  $(u-1)!$  arrangements. The numerical coefficient of this pattern is therefore

$$t^2 \cdot u^2 \cdot (t-1)! (u-1)! \quad \text{or} \quad t \cdot u \cdot t! u!,$$

while the N-coefficient is as before, and the term is that in  $\kappa_{20}^{t-1} \kappa_{02}^{u-1} \kappa_{11}^2$ , which may be written  $\sigma_1^{2t} \cdot \sigma_2^{2u} \cdot \rho^2$ .

Let now two  $\times$ 's overlap the 1's and *vice versa*. Two 1's in each column can be chosen out of  $t$  in  $\frac{t(t-1)}{2!}$  ways, and likewise two  $\times$ 's in  $\frac{u(u-1)}{2!}$  ways. There are now two quartettes

$$\begin{array}{c} 1 \\ 1 \end{array} \quad \begin{array}{c} \cdot \\ \times \end{array},$$

each of which may be arranged in two ways, remembering that the units represent different objects. Finally the blocks of 1's and  $\times$ 's left together may be arranged in  $(t-2)!$  and  $(u-2)!$  ways respectively, so that our numerical coefficient is

$$\begin{aligned} & \frac{t^2(t-1)^2}{4} \cdot \frac{u^2(u-1)^2}{4} \cdot 2.2 \cdot (t-2)! (u-2)! \\ &= \frac{t(t-1) \cdot u(u-1)}{2.2} t! u! , \end{aligned}$$

while the term is that in  $\kappa_{20}^{t-2} \kappa_{02}^{u-2} \kappa_{11}^4$ , or  $\sigma_1^{2t} \sigma_2^{2u} \rho^4$ . We have, therefore, the following series for the variance of  $k_{tu}$  :—

$$\begin{aligned} t! u! \sum_{r=1}^{t+u} \left\{ \frac{t(r-1)!}{r} \cdot \frac{\Delta^r 0^{t+u}}{N(N-1) \dots (N-r+1)} \right\} \sigma_1^{2t} \sigma_2^{2u} \left[ 1 + \frac{t \cdot u}{1.1} \rho^2 + \frac{t(t-1)u(u-1)}{1.2 \cdot 1.2} \rho^4 \right. \\ \left. + \frac{t(t-1)(t-2) \cdot u(u-1)(u-2)}{1.2.3 \cdot 1.2.3} \rho^6 + \dots \right] \end{aligned}$$

the last term being, simply,  $\frac{t!}{u!(t-u)!} \rho^{2u}$  ( $t > u$ ), corresponding to the partition where all the  $\times$ 's are partnered by 1's.

But this series is the hypergeometric series  $F(-t, -u, 1, \rho^2)$ , and we therefore have

Variance of  $k_{tu}$

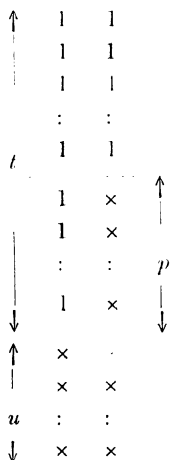
$$= t! u! \sum_{r=1}^{t+u} \left\{ \frac{t(r-1)!}{r} \cdot \frac{\Delta^r 0^{t+u}}{N(N-1) \dots (N-r+1)} \right\} \sigma_1^{2t} \sigma_2^{2u} F(-t, -u, 1, \rho^2) \quad (12),$$

in which the restriction that  $t > u$  is no longer operative.

The limitation of our patterns to those of 2 columns only, and the assumed normal law of distribution which determines that each row of the partition shall add up to  $\kappa_{20}$  or  $\kappa_{11}$ , and finally the rule that no zero entry can occur in such partitions as we are considering, preclude the possibility of any correlation existing between  $k_{tu}$  and  $k_{vw}$  unless  $v+w=t+u$ . Let us consider this case, assuming for clearness that  $w > u$ . First we note that the variance of  $k_{vw}$  is given by an expression similar

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to (12), putting  $t=v$ ,  $u=w$ . For the product semi-invariant, or moment, of  $k_{tu}$  and  $k_{vw}$  we require to consider a partition in which we have in the first column  $t$  1's and  $u$   $\times$ 's, and in the second  $v=t-p$  1's and  $w=u+p$   $\times$ 's. Consider as the first partition that in which there is as little overlap as possible.



$p$  1's can be chosen out of  $t$  in  $\frac{t!}{p!(t-p)!}$  ways, and  $p$   $\times$ 's out of  $u+p$  in  $\frac{(u+p)!}{p!u!}$  ways.  $(t-p)$  1's in each column remain partnered, and can be arranged in  $(t-p)!$  ways. Similarly the lower block containing  $u$   $\times$ 's in each column can be arranged in  $u!$  ways, and finally the central set, where  $p$  1's are partnered by  $p$   $\times$ 's, can be arranged in  $p!$  ways. The numerical coefficient is therefore

$$\frac{t!}{p!(t-p)!} \cdot \frac{(u+p)!}{p!u!} \cdot (t-p)! u! p! = \frac{t!(u+p)!}{p!},$$

while the term is that in  $\kappa_{20}^{t-p} \kappa_{02}^u \kappa_{11}^p = \sigma_1^{t+p} \sigma_2^{u+w} \rho^p$ . The N-coefficient is the same as in the case of the two variances, since it depends only on the pattern, and not at all on the arrangement within the pattern.

If we now allow another two rows to come into the "overlapping" portion we can determine in similar fashion the numerical coefficient belonging to this arrangement. It is, in fact,

$$\frac{u(t-p)(u+p)!}{(p+1)!} t!,$$

while the term is that in  $\kappa_{20}^{t-p-1} \kappa_{02}^{u-1} \kappa_{11}^{p+2} = \sigma_1^{t+v} \sigma_2^{u+w} \rho^{p+2}$ . Proceeding

in this way we find for the product semi-invariant of  $k_{tu}$  and  $k_{vw}$  the following expression

$$\frac{t!(u+p)!}{p!} \sum_{r=1}^{t+u} \left( \frac{(r-1)!}{r} \cdot \frac{\Delta^r 0^{t+u}}{N(N-1)\dots(N-r+1)} \right) \left\{ \sigma_1^{t+v} \sigma_2^{u+w} \rho^p \times \right. \\ \left. \left[ 1 + \frac{u(t-p)}{p+1} \rho^2 + \frac{u(u-1)(t-p)(t-p-1)}{1.2 \cdot (p+1)(p+2)} \rho^4 + \dots \right] \right\}$$

In this replace  $p$  by  $t-v$ , and note that the series in  $\rho^2$  is again a hypergeometric one. It becomes

$$\frac{t! \cdot u!}{(t-v)!} \sum_{r=1}^{t+u} \left( \frac{(r-1)!}{r} \cdot \frac{\Delta^r 0^{t+u}}{N(N-1)\dots(N-r+1)} \right) \left\{ \sigma_1^{t+v} \cdot \sigma_2^{u+w} \rho^{t-v} F(-u, -v, t-v+1, \rho^2) \right\} \quad (13)$$

Now take (13) in conjunction with (12), and the analogous formula for the variance of  $k_{vw}$ . We find that the correlation between  $k_{tu}$  and  $k_{vw}$  in samples of  $N$  from a normal bi-variate population of correlation  $\rho$  is

$$r_{k_{tu}, k_{vw}} = \frac{\rho^{t-v}}{(t-v)!} \cdot \frac{F(-u, -v, t-v+1, \rho^2)}{\left\{ \frac{u!}{t!} F(-t, -u, 1, \rho^2) \cdot \frac{n!}{w!} F(-v, -w, 1, \rho^2) \right\}^{\frac{1}{2}}} \quad (14)$$

This formula is subject to the conditions that  $t+u=v+w$ , while  $t-v>0$ .

A particular case of some importance occurs when  $v=u$ ,  $w=t$ . We have:

Correlation between  $k_{tu}$  and  $k_{ut}$

$$= \frac{t!}{u!(t-u)!} \rho^{t-u} \cdot \frac{F(-u, -u, t-u+1, \rho^2)}{F(-t, -u, 1, \rho^2)} \quad (t>u) \\ = \rho^{t+u} \cdot \frac{F(-t, -u, 1, 1/\rho^2)}{F(-t, -u, 1, \rho^2)} \quad (15),$$

using a well-known transformation of the hypergeometric series.

4. *Particular Cases.*—Formulæ (14) and (15) for the correlation between the symmetric functions  $k$ , as defined, are generalisations of (1). The chief interest of the practical statistician will, however, be in particular cases of low order, and for these he will require to know the actual values of the symmetric functions, in terms of the observations. Thus:

(a) Let

$$k_{21} = \frac{N^2}{(N-1)(N-2)} m_{21} \quad \text{and} \quad k_{12} = \frac{N^2}{(N-1)(N-2)} m_{12},$$

$m_{21}$  being the third order product moment estimate

$$\frac{1}{N} \sum_{i=1}^N (x - \bar{x})^2 (y - \bar{y}),$$

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so that, if we like,

$$k_{21} = \frac{N}{(N-1)(N-2)} \left( s_{21} - \frac{2}{N} s_{11} s_{10} - \frac{1}{N} s_{20} s_{01} + \frac{2}{N^2} s_{10}^2 s_{01} \right),$$

where

$$s_{ab} = \sum_1^N x^a y^b.$$

Then

$$r_{k_{21} \cdot k_{12}} = \frac{\rho(2 + \rho^2)}{1 + 2\rho^2} \quad \dots \quad (16)$$

(b) Let

$$\begin{aligned} k_{31} &= \frac{N^2}{(N-1)(N-2)(N-3)} \left\{ (N+1)m_{31} - 3(N-1)m_{20}m_{11} \right\} \\ &= \frac{N}{(N-1)(N-2)(N-3)} \left\{ (N+1)s_{31} - \frac{N+1}{N}(s_{30}s_{01} + 3s_{21}s_{10}) - \frac{3(N-1)}{N}s_{20}s_{11} \right. \\ &\quad \left. + \frac{6}{N}(s_{20}s_{10}s_{01} + s_{11}s_{10}^2) - \frac{6}{N^2}s_{10}^3s_{01} \right\}, \end{aligned}$$

with a similar expression for  $k_{13}$ . Then

$$r_{k_{31} \cdot k_{13}} = \frac{\rho^2(3 + \rho^2)}{1 + 3\rho^2} \quad \dots \quad (17)$$

(c) Let

$$\begin{aligned} k_{22} &= \frac{N^2}{(N-1)(N-2)(N-3)} \left\{ (N+1)m_{22} - (N-1)(m_{20}m_{02} + 2m_{11}^2) \right\} \\ &= \frac{N}{(N-1)(N-2)(N-3)} \left\{ (N+1)s_{22} - 2\frac{N+1}{N}(s_{21}s_{01} + s_{10}s_{12}) - \frac{N-1}{N}(s_{20}s_{02} + 2s_{11}^2) \right. \\ &\quad \left. + \frac{2}{N}(s_{20}s_{01}^2 + s_{02}s_{10}^2 + 4s_{11}s_{10}s_{01}) - \frac{6}{N^2}s_{10}^2s_{01}^2 \right\}. \end{aligned}$$

Then

$$r_{k_{31} \cdot k_{22}} = r_{k_{13} \cdot k_{22}} = \frac{\sqrt{6} \cdot \rho(1 + \rho^2)}{\{(1 + 3\rho^2)(1 + 4\rho^2 + \rho^4)\}^{\frac{1}{2}}} \quad \dots \quad (18)$$

5. To sum up, it is apparent that no simple generalisation of equations (1) and (2) exists for the correlation between the ordinary moment coefficients. A law has been found, however, for certain symmetric functions derived from the moments, and which are estimates of the semi-invariants of the population. Similar generalisations have been found already to hold for these functions in sampling problems. Thus the well-known formulæ for the moments of the distribution of the mean in samples are summed up, in the notation of this paper, by the formula

$$\kappa(1^r) = \frac{\kappa_r}{N^{r-1}},$$

or, in words: The  $r^{\text{th}}$  semi-invariant of the distribution of the mean is equal to the  $r^{\text{th}}$  semi-invariant of the sampled population, divided by

$N^{r-1}$ . Again, I have shown elsewhere (7) that a study of the number of ways of arranging different rods in a ring leads to the determination of the moments of the simultaneous distribution of second order product moments in samples from a normal multi-variate population. The moments determined directly by this combinatorial problem are, however, the semi-invariants, which can then be transformed into direct moments if desired. Finally, R. A. Fisher, in the paper cited at the outset (4), has shown that all the moments of moment functions in samples can be directly and simply determined in semi-invariant form. These interesting relationships, of which only a special class of cases has been studied here, will well repay further examination, while the convenient generalisations made possible by their means may be held to justify the departure from the traditional machinery of the English biometrician.

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#### REFERENCES.

- (1) PEARSON, K., and FILON, L. N. G., *Phil. Trans. (London)*, A, 191 (1898), p. 229.
- PEARSON, K., *Biometrika*, ii (1903), p. 273 ; ix (1913), p. 1.
- (2) "STUDENT," *Biometrika*, vi (1908), p. 1.
- (3) WISHART, J., *Biometrika*, xxa (1928), pp. 32-52.
- (4) FISHER, R. A., *Proc. Lond. Math. Soc.* (at press); *Rothamsted Memoirs*, 14.
- (5) FRISCH, R., "Sur les Semi-Invariants et Moments Employés dans L'Étude des Distributions Statistiques," *Norske Videnskaps-Akademi*, ii, 3 (1926), p. 16.
- (6) WHITTAKER and ROBINSON, *Calculus of Observations* (1924), p. 7.
- STEFFENSEN, J. F., *Interpolation* (1927), p. 55.
- (7) WISHART, J., *Proc. Lond. Math. Soc.*, (2) 29 (1929), pp. 309-321 ; *Rothamsted Memoirs*, 14.



# MOMENTS AND PRODUCT MOMENTS OF SAMPLING DISTRIBUTIONS

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## 1. *Introductory.*

If a random sample of  $n$  observations be taken from a univariate distribution, and the sample values obtained be designated by  $x_1, x_2, \dots, x_n$ , then any symmetric function of these sample values of degree  $r$  may be termed a moment function of the sample of the  $r$ -th degree. If the coefficients of the symmetric function involve the sample number  $n$  in such a way that, as  $n$  tends to infinity, the value of the function tends to a finite limit, in the sense that the probability of exceeding or falling short of that limit by a positive quantity  $\epsilon$ , however small, tends to zero, then the limit to which it tends is a moment function of the population sampled, and the moment function of the sample may be regarded as a statistical estimate of the corresponding moment function of the population.

If we consider the random sampling distribution of such a statistic it is evident that the moment functions of this distribution will be expressible in terms of the moment functions of the original distribution, in so far as these are finite, by means of formulae which will be independent of the nature of this distribution. For example, a moment function of degree  $s$  of the sampling distribution of a moment function of degree  $r$  will involve only symmetric functions of the observations of degree  $rs$ , and will therefore be expressible as a moment function of the population of this degree, irrespective of the moments of higher degree.

Numerous researches have been made into the moments, chiefly of the second order, of moment statistics. The algebraic method was developed by Sheppard [1], and used extensively by Pearson [2, 3] and Isserlis [4, 5]; in all these researches, however, owing to the supposition that the mean of the sample coincides with the mean of the population, or for other similar reasons, the results are only first approximations neglecting  $n^{-1}$ . In 1913 [6] Soper obtained a number of approximations as far as  $n^{-2}$ . In 1908 "Student" [7] derived an exact formula for the second moment of the variance as estimated, which corresponds in a different notation to equation (1) of this paper for the univariate case. Later, much work, by the exact algebraic method, was carried out by Tchouproff [8], who obtained in this way the first eight moments of the mean, in addition to the univariate formulae corresponding to numbers (5) and (14). Tchouproff's version of (14) in the univariate problem was subsequently corrected by Church [9]. The application of the combinatorial method developed below to the general moments of the distribution of statistics of the second degree from normal multivariate populations has already appeared in a paper by J. Wishart [11].

Apart from the last, these results are subject to two somewhat serious limitations; the great complexity of the results attained detracts largely from the possibility either of a theoretical comprehension of their meaning, or of numerical applications; it has also led to great difficulties in the detection of errors, which have had on more than one occasion to be corrected by subsequent workers. Secondly, partly no doubt in consequence of this complexity, attention has been almost solely confined to the direct moments of single statistics, and the product moments, specifying the simultaneous distribution of two or more statistics, have been largely neglected. The total number of formulae of degree no higher than 12 is large, and it is scarcely possible that the whole body should be made available, either for study or for use, unless an improved notation can be found which will greatly simplify the algebraic expressions. It will be shown that the formulae are much simplified by the use of the cumulative moment functions, or semi-invariants, in place of the crude moments.

The importance of the formulae lies in their generality; they are applicable to all distributions for which the expressions have a meaning. In the present state of our knowledge any information, however incomplete, as to sampling distributions is likely to be of frequent use, irrespective of the fact that moment functions only provide statistical estimates of high efficiency for a special type of distribution [10].

2. *The cumulative moment functions.*

If the probability that a single sample value falls in the range  $dx$  is

$$\phi(x) dx,$$

then the function

$$M = \int e^{tx} \phi(x) dx,$$

taken over all possible values of the variate  $x$ , may, or may not, have a meaning for real values of  $t$ . If it has a meaning we may expand the exponential term, and, writing

$$\mu_r = \int x^r \phi(x) dx,$$

we have 
$$M = 1 + \mu_1 t + \mu_2 \frac{t^2}{2!} + \mu_3 \frac{t^3}{3!} + \dots$$

If we expand the logarithm of  $M$  in powers of  $t$  we may write

$$K = \log M = \kappa_1 t + \kappa_2 \frac{t^2}{2!} + \kappa_3 \frac{t^3}{3!} + \dots,$$

where the cumulative moment functions  $\kappa$  are determinate functions of the moments  $\mu$ , whether the series converges or not; moreover, since  $\kappa_r$  involves only  $\mu_r$ , and lower orders, it follows that, if  $\mu_1, \dots, \mu_r$  are finite, so will  $\kappa_1, \dots, \kappa_r$  be finite.

The expression of  $\kappa_r$  in terms of  $\mu$  will involve the term

$$\mu_{p_1}^{\pi_1} \mu_{p_2}^{\pi_2} \dots \mu_{p_h}^{\pi_h}$$

corresponding to any partition

$$(p_1^{\pi_1} p_2^{\pi_2} \dots p_h^{\pi_h})$$

of the integer  $r$ , with coefficient

$$\frac{(-)^{\rho-1}(\rho-1)!}{\pi_1! \pi_2! \dots \pi_h!} \frac{r!}{(p_1!)^{\pi_1} (p_2!)^{\pi_2} \dots (p_h!)^{\pi_h}},$$

where  $\rho = \Sigma(\pi)$  is the number of parts.

Similarly, the expression  $\mu_r$  in terms of  $\kappa$  will involve the term

$$\kappa_{p_1}^{\pi_1} \kappa_{p_2}^{\pi_2} \dots \kappa_{p_h}^{\pi_h}$$

with coefficient

$$\frac{1}{\pi_1! \pi_2! \dots \pi_h!} \frac{r!}{(p_1!)^{\pi_1} (p_2!)^{\pi_2} \dots (p_h!)^{\pi_h}}.$$

The simplification of moment formulae obtained by referring the moments to the mean of the distribution is due to the fact that, when  $\mu_1 = 0$ , no subsequent  $\mu$  involves  $\kappa_1$ , and the number of partitions required is much reduced; thus

$$\mu_2 = \kappa_2, \quad \mu_3 = \kappa_3, \quad \mu_4 = \kappa_4 + 3\kappa_2^2,$$

and so on. The advantage of this simplification may be carried to higher orders by consistently using the cumulative moment functions  $\kappa$  in place of the moments  $\mu$ .

The cumulative moment functions supply an immediate solution of the problem of the distribution of the mean, for, using the well known cumulative property, that, if  $x$  and  $y$  are independent variates,

$$K(x+y) = K(x) + K(y),$$

where  $K(x)$  stands for the  $K$  function specifying the distribution of  $x$ , we find that, if  $s_1 = S(x)$  is the sum of  $n$  independent values constituting a sample from a given distribution, then

$$\begin{aligned} K(s_1) &= nK(x) \\ &= n\kappa_1 t + n\kappa_2 \frac{t^2}{2!} + n\kappa_3 \frac{t^3}{3!} + \dots; \end{aligned}$$

but the mean is  $\bar{x} = (1/n)s_1$ ; consequently the  $K$  function of the mean is found by substituting  $t/n$  for  $t$  in the series for  $K(s_1)$ , giving

$$K(\bar{x}) = \kappa_1 t + \frac{\kappa_2}{n} \frac{t^2}{2!} + \frac{\kappa_3}{n^2} \frac{t^3}{3!} + \dots.$$

The value of  $\kappa_r$  in the distribution of the mean is thus found from that of the sampled distribution by dividing by  $n^{r-1}$ .

### 3. *The appropriate moment statistics.*

In order to take the full advantage of the properties of the cumulative moment functions, it is necessary to introduce a modification also into the form of the moment statistics; it is usual to employ statistics which

may be written 
$$m_r = \frac{1}{n} S(x - \bar{x})^r,$$

which are called the moments of the sample about its mean, together with the mean itself,  $\bar{x}$ . These moments may be expressed in terms of the symmetric functions  $s_r$ , defined by

$$s_r = S(x^r),$$

by direct expansion; for example,

$$\bar{x} = n^{-1} s_1,$$

$$m_2 = n^{-1} s_2 - n^{-2} s_1^2,$$

$$m_3 = n^{-1} s_3 - 3n^{-2} s_1 s_2 + 2n^{-3} s_1^3,$$

and so on. While the coefficients  $n^{-1}$ ,  $n^{-2}$ , etc., are kept simple, we here encounter the complication that the mean value of  $m_s$  is not in finite samples equal to  $\mu_s$ ; in order that this should be so we should multiply  $m_2$  by  $n/(n-1)$ , and  $m_3$  by  $n^2/[(n-1)(n-2)]$ ; further, for functions of the fourth and higher degrees,  $\kappa_r$  is not a linear function of the moments  $\mu_r$ , and, in consequence, a moment statistic of which the mean is  $\kappa_r$  will not be exactly the same function of moment statistics, of which the means are  $\mu_r$ , as  $\kappa_r$  is of  $\mu_r$ . As a preliminary step, therefore, to the simplification of the formulæ to be obtained, it will be desirable to obtain, in terms of the direct summation values  $s_r$ , the moment statistics of each degree of which the sampling means shall be  $\kappa_1$ ,  $\kappa_2$ ,  $\kappa_3$ , .... They will be represented by  $k_1$ ,  $k_2$ ,  $k_3$ , ....

The first few statistics which fulfil this condition are

$$k_1 = m_1 = n^{-1} s_1,$$

$$k_2 = \frac{n}{n-1} m_2 = \frac{1}{n-1} (s_2 - n^{-1} s_1^2),$$

$$k_3 = \frac{n^2}{(n-1)(n-2)} m_3 = \frac{n}{(n-1)(n-2)} (s_3 - 3n^{-1} s_1 s_2 + 2n^{-2} s_1^3),$$

$$\begin{aligned} k_4 &= \frac{n^3}{(n-1)(n-2)(n-3)} \{ (n+1) m_4 - 3(n-1) m_2^2 \} \\ &= \frac{n}{(n-1)(n-2)(n-3)} \{ (n+1) s_4 - 4n^{-1}(n+1) s_1 s_3 - 3n^{-1}(n-1) s_2^2 \\ &\quad + 12n^{-1} s_1^2 s_2 - 6n^{-2} s_1^4 \}, \end{aligned}$$

$$\begin{aligned}
k_5 &= \frac{n^3}{(n-1)(n-2)(n-3)(n-4)} \{ (n+5)m_5 - 10(n-1)m_2m_3 \} \\
&\quad - \frac{n^2}{(n-1)(n-2)(n-3)(n-4)} \\
&\quad \times \left\{ (n+5)s_5 - 5 \frac{n+5}{n} s_1s_4 - 10 \frac{n-1}{n} s_2s_3 \right. \\
&\quad \left. + 20 \frac{n+2}{n^2} s_1^2s_3 + 30 \frac{n-1}{n^2} s_1s_2^2 - \frac{60}{n^2} s_1^3s_2 + \frac{24}{n^3} s_1^3 \right\}, \\
k_6 &= \frac{n^2}{(n-1) \dots (n-5)} \{ (n+1)(n^2+15n-4)m_6 - 15(n-1)^2(n+4)m_2m_4 \\
&\quad - 10(n-1)(n^2-n+4)m_3^2 + 30n(n-1)(n-2)m_2^3 \} \\
&= \frac{n}{(n-1) \dots (n-5)} \left\{ (n+1)(n^2+15n-4)s_6 - 6 \frac{n+1}{n} (n^2+15n-4)s_1s_5 \right. \\
&\quad - 15 \frac{(n-1)^2}{n} (n+4)s_2s_4 - 10 \frac{n-1}{n} (n^2-n+4)s_2^2 \\
&\quad + 30 \frac{n^2+9n+2}{n} s_1s_4 + 120 \frac{n^2-1}{n} s_1s_3s_3 \\
&\quad + 30 \frac{(n-1)(n-2)}{n} s_2^3 - 120 \frac{n+3}{n} s_1^3s_3 \\
&\quad \left. - 270 \frac{n-1}{n} s_1^2s_2^2 + \frac{360}{n} s_1^4s_2 - \frac{120}{n^2} s_1^6 \right\}.
\end{aligned}$$

If these be employed we have not only the result that the  $r$ -th cumulative moment function of the mean is  $n^{-(r-1)}\kappa_r$ , but also that the mean of  $k_r$  is  $\kappa_r$ , thus reducing a second group of the required formulae to its simplest form. It is, however, the effect of their use upon the more complex formulae which is of the greater importance. The general structure of  $k$  for any degree will be elucidated in § 10.

#### 4. The aggregate of moment sampling formulae.

If we consider in its full generality the simultaneous distribution in random samples of the statistics  $k_1, k_2, k_3, \dots$ , it is clear that we can represent it by means of cumulative moment functions analogous to those

developed for a single variate. To any partition

$$(p_1^{\pi_1} p_2^{\pi_2} \dots p_h^{\pi_h})$$

of the number  $r$ , there will correspond a moment

$$\mu(p_1^{\pi_1} p_2^{\pi_2} \dots p_h^{\pi_h}) = \text{mean value of } k_{p_1}^{\pi_1} k_{p_2}^{\pi_2} \dots k_{p_h}^{\pi_h},$$

and, if we write

$$M = \Sigma \mu(p_1^{\pi_1} p_2^{\pi_2} \dots p_h^{\pi_h}) \frac{t_1^{\pi_1}}{\pi_1!} \frac{t_2^{\pi_2}}{\pi_2!} \dots \frac{t_h^{\pi_h}}{\pi_h!},$$

the expansion in terms of  $t_1, t_2, \dots$  of  $K = \log M$  assumes the form

$$K = \Sigma \kappa(p_1^{\pi_1} p_2^{\pi_2} \dots p_h^{\pi_h}) \frac{t_1^{\pi_1}}{\pi_1!} \frac{t_2^{\pi_2}}{\pi_2!} \dots \frac{t_h^{\pi_h}}{\pi_h!}.$$

There will thus be a separate formula of degree  $r$  for every partition of the number  $r$ , and for the complete specification of the distribution each must be expanded in terms of the cumulative moment functions of the sampled population. For example, the semi-invariants of the distribution of the second moment statistic  $k_2$  will be given by the terms corresponding to the partitions (2), (2<sup>2</sup>), (2<sup>3</sup>), (2<sup>4</sup>), ..., which we designate by

$$\kappa(2), \kappa(2^2), \kappa(2^3), \kappa(2^4), \text{ and so on.}$$

The well known solution of the distribution of the mean, given above, may now be written

$$\kappa(1^r) = \frac{\kappa_r}{n^{r-1}}, \quad (I)$$

while from the manner in which the statistics  $k$  have been constructed we have also

$$\kappa(r) = \kappa_r. \quad (II)$$

In general, the expression for the  $\kappa$  corresponding to any given partition of  $r$  will include a term in  $\kappa_r$  together with terms of the form

$$A(q_1^{x_1} q_2^{x_2} \dots q_h^{x_h}) \kappa_{q_1}^{x_1} \kappa_{q_2}^{x_2} \dots \kappa_{q_h}^{x_h},$$

where  $q_1^{x_1} q_2^{x_2} \dots q_h^{x_h}$  is any partition of  $r$  in which no part is unity. This restriction, which greatly diminishes the number of terms to be evaluated, flows from the consideration that  $\kappa_1$ , unlike all other cumulative moment functions, is altered by a change of origin, and by such a change can be given any desired value, while of the moment statistics

also  $k_1$  is the only one affected by such a change, and that by addition of a quantity which is invariable from sample to sample; consequently,  $\kappa_1$  can only appear in the single formula

$$\kappa(1) = \kappa_1,$$

expressing that the mean of the sample of  $n$  will be the mean of the population.

### 5. Partitions involving unit parts.

A relationship exists, of which a proof may be deduced from the general theory to be developed, which enables us to dispense with the separate examination and tabulation of the formulae corresponding to all those partitions which involve unit parts. The effect upon the corresponding formula of adding a new unit part to the partition is (1) to modify every term in the formula by increasing the suffix of one of its  $\kappa$  functions by unity in every possible way, and (2) to divide the whole by  $n$ . For example, the formula for the variance of  $k_2$  is

$$\kappa(2^2) = \frac{1}{n} \kappa_4 + \frac{2}{n-1} \kappa_2^2,$$

whence we may deduce, by applying the above rules,

$$\kappa(2^2 1) = \frac{1}{n^2} \kappa_5 + \frac{4}{n(n-1)} \kappa_2 \kappa_3,$$

and, by further applications,

$$\kappa(2^2 1^2) = \frac{1}{n^3} \kappa_6 + \frac{4}{n^2(n-1)} \kappa_2 \kappa_4 + \frac{4}{n^2(n-1)} \kappa_3^2,$$

$$\kappa(2^2 1^3) = \frac{1}{n^4} \kappa_7 + \frac{4}{n^3(n-1)} \kappa_2 \kappa_5 + \frac{12}{n^3(n-1)} \kappa_3 \kappa_4,$$

and so on.

An immediate consequence of the same relationship is that

$$\kappa(r 1^s) = \frac{\kappa_{r+s}}{n^s} = \frac{1}{n^s} \kappa_{r+s}. \quad (\text{III})$$

The number of formulae remaining of any degree  $r$  is the number

of partitions of  $r$  into parts of 2 or more; these are

$r$	4	5	6	7	8	9	10	11	12	13	14	15	16	17
partitions	1	1	3	3	6	7	11	13	20	23	33	40	54	65

Up to the 12th degree there are therefore 65 formulae, while 150 more will only reach the 16th degree. It is proposed to put on record, as a basis for discussion, the formulae up to the 10th degree, together with a few others of special interest, with an explanation of the procedure of calculation.

### 6. Calculation of formulae.

In the calculation of the formulae by the algebraic method it is desirable to proceed somewhat formally, although the results for the 4th and 5th degrees may be obtained fairly readily by writing down the algebraical expressions at length. The procedure may be illustrated by the work for the formulae of the eighth degree. There will be six of these, and corresponding to any of these, such as  $\kappa(62)$ , the  $k$  product,  $k_6 k_2$ , may be written down and expanded in the symmetric functions  $s$ . The work proceeds in three steps: (1) the mean value of the  $k$  product is expressed in terms of the population moments  $\mu$ ; (2) by substitution, the expression in terms of  $\mu$  is condensed into its equivalent in terms of  $\kappa$ ; (3) from the moment thus obtained, corresponding to the required partition, the corresponding cumulative moment function is found by the use of formulae of lower degree previously prepared.

The first step is carried out by means of easily verified relationships giving the mean value of such a product as  $s_p s_q s_r$  in the form

$$n\mu_{p+q+r} + n(n-1)(\mu_p\mu_{q+r} + \mu_q\mu_{r+p} + \mu_r\mu_{p+q}) + n(n-1)(n-2)\mu_p\mu_q\mu_r.$$

In order to apply these relationships expeditiously a table is prepared for each degree, showing the coefficients with which each  $\mu$  product, ignoring  $\mu_1$ , occurs in the expansion of each  $s$  product.

To evaluate the mean value of any  $k$  product, such as  $k_8^2 k_2$ , it is first expanded in  $s$  products as

$$\frac{n^2}{(n-1)^3(n-2)^2} \left( s_8^2 s_2 - \frac{1}{n} s_8^2 s_1^2 - \frac{6}{n} s_8 s_2^2 s_1 + \frac{10}{n^2} s_8 s_2 s_1^3 + \frac{9}{n^2} s_2^3 s_1^2 - \frac{4}{n^3} s_8 s_1^5 \right. \\ \left. - \frac{21}{n^3} s_2^2 s_1^4 + \frac{16}{n^4} s_2 s_1^6 - \frac{4}{n^5} s_1^8 \right),$$

whence from a table of the separations of 8 the following table may at once be constructed.

TABLE 1.  
Calculation of the mean value of  $k_3^2 k_2$ .

	$n(n-1)$	$n(n-1)$	$n(n-1)$	$n(n-1)(n-2)$	$n(n-1)(n-2)$	$n(n-1)(n-2)(n-3)$	
	$n\mu_6$	$\mu_6\mu_2$	$\mu_5\mu_3$	$\mu_4^2$	$\mu_4\mu_2^2$	$\mu_3^2\mu_2$	$\mu_2^4$
$s_3^2 s_2$	1	1	2	—	—	1	—
$s_3^2 s_1^2$	$n^{-1} \left\{ \begin{array}{l} -1 \\ -6 \end{array} \right.$	$\left\{ \begin{array}{l} -1 \\ -12 \end{array} \right.$	$\left\{ \begin{array}{l} -2 \\ -18 \end{array} \right.$	$\left\{ \begin{array}{l} 2 \\ -8 \end{array} \right.$	$\left\{ \begin{array}{l} — \\ -8 \end{array} \right.$	$\left\{ \begin{array}{l} -1 \\ -12 \end{array} \right.$	$\left\{ \begin{array}{l} — \\ — \end{array} \right.$
$s_3 s_2^2 s_1$	$n^{-2} \left\{ \begin{array}{l} 10 \\ 9 \end{array} \right.$	$\left\{ \begin{array}{l} 40 \\ 36 \end{array} \right.$	$\left\{ \begin{array}{l} 50 \\ 54 \end{array} \right.$	$\left\{ \begin{array}{l} 30 \\ 27 \end{array} \right.$	$\left\{ \begin{array}{l} 30 \\ 54 \end{array} \right.$	$\left\{ \begin{array}{l} 40 \\ 54 \end{array} \right.$	$\left\{ \begin{array}{l} — \\ 9 \end{array} \right.$
$s_3 s_2 s_1^2$	$n^{-3} \left\{ \begin{array}{l} -4 \\ -21 \end{array} \right.$	$\left\{ \begin{array}{l} -40 \\ -168 \end{array} \right.$	$\left\{ \begin{array}{l} -44 \\ -252 \end{array} \right.$	$\left\{ \begin{array}{l} -20 \\ -147 \end{array} \right.$	$\left\{ \begin{array}{l} -60 \\ -336 \end{array} \right.$	$\left\{ \begin{array}{l} -40 \\ -420 \end{array} \right.$	$\left\{ \begin{array}{l} — \\ -63 \end{array} \right.$
$s_3 s_1^5$	$n^{-4} \left( \begin{array}{l} 16 \\ 1 \end{array} \right.$	$\left( \begin{array}{l} 256 \\ -4 \end{array} \right.$	$\left( \begin{array}{l} 416 \\ -224 \end{array} \right.$	$\left( \begin{array}{l} 240 \\ -140 \end{array} \right.$	$\left( \begin{array}{l} 960 \\ -840 \end{array} \right.$	$\left( \begin{array}{l} 1120 \\ -1120 \end{array} \right.$	$\left( \begin{array}{l} 240 \\ -420 \end{array} \right.$
$s_2 s_1^6$	$n^{-5} \left( \begin{array}{l} -4 \\ 1 \end{array} \right.$	$\left( \begin{array}{l} -112 \\ -4 \end{array} \right.$	$\left( \begin{array}{l} -224 \\ -140 \end{array} \right.$	$\left( \begin{array}{l} -140 \\ -140 \end{array} \right.$	$\left( \begin{array}{l} -840 \\ -840 \end{array} \right.$	$\left( \begin{array}{l} -1120 \\ -1120 \end{array} \right.$	$\left( \begin{array}{l} -420 \\ -420 \end{array} \right.$

Collecting like terms and cancelling the factors  $n-1$  and  $n-2$  whenever possible, we get

$$\begin{aligned} \mu(3^2 2) = & \frac{\mu_8}{n^2} + \frac{n^2 - 8n + 28}{n^2(n-1)} \mu_6 \mu_2 + \frac{2n^3 - 12n^2 + 48n - 56}{n^2(n-1)^2} \mu_5 \mu_3 \\ & + \frac{-8n^2 + 25n - 35}{n^2(n-1)^2} \mu_4^2 + \frac{1}{n^2(n-1)^2(n-2)} \\ & \times \{ (-6n^4 + 84n^3 - 396n^2 + 960n - 840) \mu_4 \mu_2^2 \\ & \quad + (n^5 - 13n^4 + 94n^3 - 460n^2 + 1120n + 1120) \mu_3^2 \mu_2 \\ & \quad + (9n^4 - 90n^3 + 429n^2 - 1140n + 1260) \mu_2^4 \}. \end{aligned}$$

The second step consists in substituting

$$\mu_4 = \kappa_4 + 3\kappa_2^2,$$

$$\mu_5 = \kappa_5 + 10\kappa_2 \kappa_3,$$

$$\mu_6 = \kappa_6 + 15\kappa_4 \kappa_2 + 10\kappa_3^2 + 15\kappa_2^3,$$

$$\mu_8 = \kappa_8 + 28\kappa_6 \kappa_2 + 56\kappa_5 \kappa_3 + 35\kappa_4^2 + 210\kappa_4 \kappa_2^2 + 280\kappa_3^2 \kappa_2 + 105\kappa_2^4,$$

which reduces the expression to the simpler form

$$\begin{aligned} \mu(3^2 2) = & \frac{\kappa_8}{n^2} + \frac{n+20}{n(n-1)} \kappa_6 \kappa_2 + \frac{2n^2 + 44n - 64}{n(n-1)^2} \kappa_5 \kappa_3 + \frac{27n - 45}{n(n-1)^2} \kappa_4^2 \\ & + \frac{9n^2 + 81n - 180}{(n-1)^2(n-2)} \kappa_4 \kappa_2^2 + \frac{n^3 + 17n^2 + 104n - 320}{(n-1)^2(n-2)} \kappa_3^2 \kappa_2 \\ & + \frac{6n^2 + 30n}{(n-1)^2(n-2)} \kappa_2^4. \end{aligned}$$

The third stage consists in removing from  $\mu(3^22)$  those terms which do not belong to  $\kappa(3^22)$ ; from the general relationship which connects these two groups of functions

$$\mu(3^22) = \kappa(3^22) + 2\kappa_3\kappa(32) + \kappa_2\kappa(3^2) + \kappa_3^2\kappa_2,$$

and from formulae of lower degree already evaluated we know that

$$\kappa(32) = \frac{\kappa_2}{n} + \frac{6\kappa_2\kappa_3}{n-1},$$

while 
$$\kappa(3^2) = \frac{\kappa_6}{n} + \frac{9\kappa_3\kappa_4}{n-1} + \frac{9\kappa_3^2}{n-1} + \frac{6n\kappa_2^3}{(n-1)(n-2)}.$$

Removing the superfluous terms we are left with

$$\begin{aligned} \kappa(3^22) = & \frac{\kappa_8}{n^2} + \frac{21}{n(n-1)}\kappa_6\kappa_2 + \frac{6(8n-11)}{n(n-1)^2}\kappa_5\kappa_3 + \frac{9(3n-5)}{n(n-1)^2}\kappa_4^2 \\ & + \frac{18(6n-11)}{(n-1)^2(n-2)}\kappa_1\kappa_2^2 + \frac{18(9n-20)}{(n-1)^2(n-2)}\kappa_3^2\kappa_2 + \frac{36n}{(n-1)^3(n-2)}\kappa_2^4, \end{aligned}$$

an expression in which the part played by each of the characteristic coefficients of the original distribution is clearly apparent. In the normal distribution, for example, when every coefficient beyond  $\kappa_2$  vanishes, only the last term remains to be evaluated.

### 7. The univariate formulae.

In addition to the partitions involving unit parts, which have already been set aside, the numbers 4 and 5 have only one partition each, 6 and 7 have three partitions each, while 8, 9, and 10 bring the total up to 32. These are given in the following Table. Since it is scarcely to be hoped that all of these, especially the heavier formulae, will be entirely free from error, it should be particularly noted that any suspected term may be evaluated separately and independently by means of the combinatorial method elaborated below. I am indebted to Dr. J. Wishart and Prof. Hotelling for checking these formulae.

In addition to these formulae, which are complete up to the tenth degree, four others of the twelfth degree may be put on record, namely those for the variance of  $k_6$ , the third moment of  $k_4$ , fourth moment of  $k_3$ ,

## TABLE OF FORMULAE.

The 32 univariate formulae up to the 10-th degree.

$\kappa(2^2)$	$\frac{\kappa_4}{n}$ 1	$\frac{\kappa_2^2}{n-1}$ 2			
$\kappa(3^2)$	$\frac{\kappa_5}{n}$ 1	$\frac{\kappa_3 \kappa_2}{n-1}$ 6			
$\kappa(4^2)$ $\kappa(3^2)$	$\frac{\kappa_6}{n}$ 1 1	$\frac{\kappa_4 \kappa_2}{n-1}$ 8 9	$\frac{\kappa_1^2}{n-1}$ 6 9	$\frac{n \kappa_2^3}{(n-1)(n-2)}$ — 6	
$\kappa(2^3)$	$\frac{\kappa_6}{n}$ 1	$\frac{\kappa_4 \kappa_2}{n(n-1)}$ 12	$\frac{\kappa_3^2}{n(n-1)^2}$ $4(n-2)$	$\frac{\kappa_2^3}{(n-1)^2}$ 8	
$\kappa(5^2)$ $\kappa(4^3)$	$\frac{1}{n} \kappa_7$ 1 1	$\frac{1}{n-1} \kappa_5 \kappa_2$ 10 12	$\frac{1}{n-1} \kappa_4 \kappa_3$ 20 30	$\frac{n}{(n-1)(n-2)} \kappa_3 \kappa_2^2$ — 36	
$\kappa(3^2^2)$	$\frac{\kappa_7}{n^2}$ 1	$\frac{\kappa_5 \kappa_3}{n(n-1)}$ 16	$\frac{\kappa_4 \kappa_3}{n(n-1)^2}$ $12(2n-3)$	$\frac{\kappa_2^2 \kappa_3}{(n-1)^2}$ 48	
$\kappa(6^2)$ $\kappa(5^3)$ $\kappa(4^2)$	$\frac{1}{n} \kappa_8$ 1 1 1	$\frac{1}{n-1} \kappa_6 \kappa_2$ 12 15 16	$\frac{1}{n-1} \kappa_5 \kappa_3$ 30 45 48	$\frac{1}{n-1} \kappa_4^2$ 20 30 34	$\frac{n}{(n-1)(n-2)} \kappa_4 \kappa_2^2$ — 60 72
$\kappa(4^2^2)$ $\kappa(3^2^2)$	$\frac{1}{n} \kappa_8$ 1 1	$\frac{\kappa_6 \kappa_2}{n(n-1)}$ 20 21	$\frac{\kappa_5 \kappa_3}{n(n-1)^2}$ $8(5n-7)$ $6(8n-11)$	$\frac{\kappa_4^2}{n(n-1)^2}$ $4(7n-10)$ $9(3n-5)$	$\frac{\kappa_4 \kappa_2^2}{(n-1)^2(n-2)}$ $80(n-2)$ $18(6n-11)$
$\kappa(2^4)$	$\frac{\kappa_8}{n^3}$ 1	$\frac{\kappa_6 \kappa_2}{n^2(n-1)}$ 24	$\frac{\kappa_5 \kappa_3}{n^2(n-1)^2}$ 32	$\frac{\kappa_4^2}{n^2(n-1)^2}$ $8(4n^2-9n+6)$	$\frac{\kappa_4 \kappa_2^2}{n(n-1)^2}$ 144
$\kappa(7^2)$ $\kappa(6^3)$ $\kappa(5^4)$	$\frac{\kappa_{11}}{n}$ 1 1 1	$\frac{\kappa_7 \kappa_2}{n-1}$ 14 18 20	$\frac{\kappa_6 \kappa_3}{n-1}$ 42 63 70	$\frac{\kappa_5 \kappa_4}{n-1}$ 70 105 120	$\frac{n \kappa_5 \kappa_2^2}{(n-1)(n-2)}$ — 90 120
$\kappa(5^2^2)$ $\kappa(4^3^2)$ $\kappa(3^3)$	$\frac{\kappa_9}{n^2}$ 1 1 1	$\frac{\kappa_7 \kappa_2}{n(n-1)}$ 24 26 27	$\frac{\kappa_6 \kappa_3}{n(n-1)^2}$ $20(3n-4)$ $24(3n-4)$ $27(3n-4)$	$\frac{\kappa_5 \kappa_4}{n(n-1)^2}$ $20(5n-7)$ $10(11n-17)$ $27(4n-7)$	$\frac{\kappa_5 \kappa_2^2}{(n-1)^2(n-2)}$ $120(n-2)$ $36(5n-9)$ $54(4n-7)$
$\kappa(3^2^3)$	$\frac{\kappa_9}{n^3}$ 1	$\frac{\kappa_7 \kappa_2}{n^2(n-1)}$ 30	$\frac{\kappa_6 \kappa_3}{n^2(n-1)^2}$ $2(31n-58)$	$\frac{\kappa_5 \kappa_4}{n^2(n-1)^2}$ $12(9n^2-23n+16)$	$\frac{\kappa_5 \kappa_2^2}{n(n-1)^2}$ 240

			(1)
			(2)
			(3)
			(4)
			(5)
			(6)
			(7)
			(8)
$\frac{n}{(n-1)(n-2)} \kappa_3^2 \kappa_2$	$\frac{n(n+1)}{(n-1)(n-2)(n-3)} \kappa_2^4$		(9)
—	—		(10)
90	—		(11)
144	24		
$\frac{\kappa_3^2 \kappa_2}{(n-1)^2(n-2)}$	$\frac{\kappa_2^4}{(n-1)^2(n-2)}$		(12)
120(n-2)	—		(13)
18(9n-20)	36n		
$\frac{\kappa_3^2 \kappa_2}{2(n-1)^3}$	$\frac{\kappa_2^4}{(n-1)^3}$		(14)
96(n-2)	48		
$\frac{n\kappa_4 \kappa_3 \kappa_2}{(n-1)(n-2)}$	$\frac{n\kappa_4^3}{(n-1)(n-2)}$	$\frac{n(n+1)\kappa_3 \kappa_2^3}{(n-1)(n-2)(n-3)}$	(15)
—	—	—	(16)
360	90	—	(17)
600	180	240	
$\frac{\kappa_4 \kappa_3 \kappa_2}{(n-1)^2(n-2)}$	$\frac{\kappa_3^3}{(n-1)^2(n-2)^2}$	$\frac{n\kappa_3 \kappa_2^3}{(n-1)^2(n-2)^2}$	(18)
480(n-2)	120(n-2)^2	—	(19)
12(61n-128)	86(n-2)(5n-12)	360(n-2)	(20)
162(5n-12)	36(7n^2-30n+34)	108(5n-12)	
$\frac{\kappa_4 \kappa_3 \kappa_2}{n(n-1)^3}$	$\frac{\kappa_3^3}{n(n-1)^3}$	$\frac{\kappa_3 \kappa_2^3}{(n-1)^3}$	(21)
360(2n-3)	24(5n-12)	336	

TABLE OF FORMULAE—continued.

	$\frac{\kappa_{10}}{n}$	$\frac{\kappa_8 \kappa_2}{n-1}$	$\frac{\kappa_7 \kappa_3}{n-1}$	$\frac{\kappa_6 \kappa_4}{n-1}$	$\frac{\kappa_5^2}{n-1}$	$\frac{n \kappa_5 \kappa_2^2}{(n-1)(n-2)}$	$\frac{n \kappa_5 \kappa_3 \kappa_2}{(n-1)(n-2)}$
$\kappa(82)$	1	16	56	112	70	—	—
$\kappa(78)$	1	21	84	168	105	126	630
$\kappa(64)$	1	24	96	194	120	180	1080
$\kappa(5^2)$	1	25	100	200	125	200	1200
	$\frac{\kappa_{10}}{n^2}$	$\frac{\kappa_8 \kappa_2}{n-1}$	$\frac{\kappa_7 \kappa_3}{n(n-1)^2}$	$\frac{\kappa_6 \kappa_4}{n(n-1)^2}$	$\frac{\kappa_5^2}{n(n-1)^2}$	$\frac{\kappa_6 \kappa_2^2}{(n-1)^2(n-1)}$	$\frac{\kappa_5 \kappa_3 \kappa_2}{(n-1)^2(n-2)}$
$\kappa(62^2)$	1	28	12(7n-9)	4(41n-56)	20(5n-7)	168(n-2)	840(n-2)
$\kappa(592)$	1	31	101n-131	5(37n-55)	5(23n-35)	30(9n-16)	30(45n-92)
$\kappa(4^22)$	1	32	8(13n-17)	4(49n-73)	4(23n-46)	8(37n-65)	1536(n-2)
$\kappa(43^2)$	1	33	6(19n-25)	3(65n-107)	6(19n-34)	18(19n-33)	72(23n-52)
	$\frac{\kappa_{10}}{n^3}$	$\frac{\kappa_8 \kappa_2}{n^2(n-1)}$	$\frac{\kappa_7 \kappa_3}{n^2(n-1)^2}$	$\frac{\kappa_6 \kappa_4}{n^2(n-1)^3}$	$\frac{\kappa_5^2}{n^2(n-1)^3}$	$\frac{\kappa_6 \kappa_2^2}{n(n-1)^2(n-2)}$	$\frac{\kappa_5 \kappa_3 \kappa_2}{n(n-1)^3(n-2)}$
$\kappa(42^3)$	1	36	4(23n-37)	4(47n^2-120n+81)	12(9n^2-24n+17)	360(n-2)	288(5n-7)(n-2)
$\kappa(3^22^2)$	1	37	6(17n-27)	3(61n^2-166n+117)	2(59n^2-154n+113)	6(67n-131)	24(71n^2-246n+202)
	$\frac{\kappa_{10}}{n^4}$	$\frac{\kappa_8 \kappa_2}{n^3(n-1)}$	$\frac{\kappa_7 \kappa_3}{n^3(n-1)^2}$	$\frac{\kappa_6 \kappa_4}{n^3(n-1)^3}$	$\frac{\kappa_5^2}{n^3(n-1)^4}$	$\frac{\kappa_6 \kappa_2^2}{n^2(n-1)^2}$	$\frac{\kappa_5 \kappa_3 \kappa_2}{n^2(n-1)^3}$
$\kappa(2^5)$	1	40	80(n-2)	40(5n^2-12n+9)	16(n-2)(6n^2-12n+17)	480	1280(n-2)

and the sixth moment of  $k_2$ . These are :—

$$\begin{aligned}
 \kappa(6^2) = & \frac{1}{n} \kappa_{12} + \frac{1}{n-1} (36\kappa_{10}\kappa_2 + 180\kappa_9\kappa_3 + 465\kappa_8\kappa_4 + 780\kappa_7\kappa_5 + 461\kappa_6^2) \\
 & + \frac{n}{(n-1)(n-2)} (450\kappa_8\kappa_3^2 + 3600\kappa_7\kappa_3\kappa_2 + 7200\kappa_6\kappa_4\kappa_2 + 6300\kappa_6\kappa_3^2 \\
 & \quad + 4500\kappa_5^2\kappa_2 + 21600\kappa_5\kappa_4\kappa_3 + 4950\kappa_4^3) \\
 & + \frac{n(n+1)}{(n-1)(n-2)(n-3)} (2400\kappa_6\kappa_2^2 + 21600\kappa_5\kappa_3\kappa_2^2 \\
 & \quad + 15300\kappa_4^2\kappa_2^2 + 54000\kappa_4\kappa_3^2\kappa_2 + 8100\kappa_3^4) \\
 & + \frac{n^2(n+5)}{(n-1)(n-2)(n-3)(n-4)} (5400\kappa_4\kappa_2^4 + 21600\kappa_3^3\kappa_2^3) \\
 & + \frac{n(n+1)(n^2+15n-4)}{(n-1)(n-2)(n-3)(n-4)(n-5)} 720\kappa_2^6. \quad (50)
 \end{aligned}$$

$$\begin{aligned}
 \kappa(4^3) = & \frac{1}{n^2} \kappa_{12} + \frac{48}{n(n-1)} \kappa_{10}\kappa_2 + \frac{16(13n-17)}{n(n-1)^2} \kappa_9\kappa_3 + \frac{12(41n-65)}{n(n-1)^2} \kappa_8\kappa_4 \\
 & + \frac{48(16n-29)}{n(n-1)^2} \kappa_7\kappa_5 + \frac{12(37n-70)}{n(n-1)^2} \kappa_6^2 + \frac{72(11n-19)}{(n-1)^2(n-2)} \kappa_8\kappa_2^2 + \dots (\text{p. 213})
 \end{aligned}$$

$\frac{n\kappa_1^2\kappa_2}{(n-1)(n-2)}$	$\frac{n\kappa_1\kappa_2^2}{(n-1)(n-2)}$	$\frac{n(n+1)\kappa_1\kappa_2^3}{(n-1)(n-2)(n-3)}$	$\frac{n(n+1)\kappa_1^2\kappa_2^2}{(n-1)(n-2)(n-3)}$	$\frac{n^2(n+5)\kappa_2^2}{(n-1)(n-2)(n-3)(n-4)}$	(22)
—	—	—	—	—	(23)
420	690	—	—	—	(24)
720	1260	480	1080	—	(25)
850	1200	600	1800	120	
$\frac{\kappa_1^2\kappa_2}{(n-1)^2(n-2)}$	$\frac{\kappa_1\kappa_2^2}{(n-1)^2(n-2)}$	$\frac{\kappa_1\kappa_2}{(n-1)^2(n-2)}$	$\frac{\kappa_2^2\kappa_2'}{(n-1)^2(n-2)}$	$\frac{n\kappa_2^2}{(n-1)^2(n-2)}$	(26)
560(n-2)	840(n-2)	—	—	—	(27)
60(15n-31)	30(45n-103)	720n	1620n	—	
144(7n-15)	72(21n-50)	$\frac{96(10n^2-27n-1)}{n-3}$	$\frac{144(17n^2-53n-2)}{n-3}$	$\frac{192(n+1)}{n-3}$	(28)
54(19n-48)	$\frac{54(33n^2-148n+172)}{n-2}$	$\frac{72n(17n-40)}{n-2}$	$\frac{108n(27n-70)}{n-2}$	$\frac{216n}{n-2}$	(29)
$\frac{\kappa_1^2\kappa_2}{n(n-1)^2(n-2)}$	$\frac{\kappa_1\kappa_2^2}{n(n-1)^2(n-2)}$	$\frac{\kappa_1\kappa_2^3}{(n-1)^3(n-2)}$	$\frac{\kappa_2^2\kappa_2'}{(n-1)^3(n-2)}$	$\frac{n\kappa_2^2}{(n-1)^3(n-2)}$	(30)
144(7n-10)(n-2)	24(49n-95)(n-2)	768(n-2)	1872(n-2)	—	(31)
36(29n^2-103n+93)	36(38n^2-155n+160)	72(14n-23)	144(19n-44)	288	
$\frac{\kappa_1^2\kappa_2}{n^2(n-1)^4}$	$\frac{\kappa_1\kappa_2^2}{n^2(n-1)^4}$	$\frac{\kappa_1\kappa_2^3}{n(n-1)^3}$	$\frac{\kappa_2^2\kappa_2'}{n(n-1)^3}$	$\frac{\kappa_2^2}{(n-1)^4}$	(32)
320(4n^2-9n+6)	480(2n^2-7n+6)	1920	1920(n-2)	384	

$$\begin{aligned}
& + \frac{288(19n-41)}{(n-1)^2(n-2)} \kappa_7 \kappa_3 \kappa_2 + \frac{48(203n-523)}{(n-1)^2(n-2)} \kappa_6 \kappa_4 \kappa_2 \\
& + \frac{144(56n^2-257n+302)}{(n-1)^2(n-2)^2} \kappa_6 \kappa_3^2 + \frac{1440(4n-11)}{(n-1)^2(n-2)} \kappa_5^2 \kappa_2 \\
& + \frac{1152(22n^2-106n+133)}{(n-1)^2(n-2)^2} \kappa_5 \kappa_4 \kappa_3 + \frac{8(709n^2-3430n+4456)}{(n-1)^2(n-2)^2} \kappa_4^3 \\
& + \frac{288(19n^3-98n^2+125n+2)}{(n-1)^2(n-2)^2(n-3)} \kappa_6 \kappa_2^3 + \frac{1728(21n^3-119n^2+164n+4)}{(n-2)^2(n-2)^2(n-3)} \kappa_5 \kappa_3 \kappa_2^2 \\
& + \frac{432(49n^3-287n^2+408n+12)}{(n-1)^2(n-2)^2(n-3)} \kappa_4^2 \kappa_2^2 + \frac{864(103n^3-629n^2+984n+24)}{(n-1)^2(n-2)^2(n-3)} \kappa_4 \kappa_3^2 \kappa_2 \\
& + \frac{288(41n^4-384n^3+1209n^2-1282n-36)}{(n-1)^2(n-2)^2(n-3)^2} \kappa_3^4 + \frac{288(89n^2-323n-88)n}{(n-1)^2(n-2)^2(n-3)} \kappa_4 \kappa_2^4 \\
& + \frac{1728(29n^3-196n^2+317n+62)n}{(n-1)^2(n-2)^2(n-3)^2} \kappa_3^2 \kappa_2^2 + \frac{1728(n^2-5n+2)(n+1)n}{(n-1)^2(n-2)^2(n-3)^2} \kappa_2^5, \quad (57)
\end{aligned}$$

$$\begin{aligned}
\kappa(3^4) = & \frac{1}{n^3} \kappa_{12} + \frac{54}{n^2(n-1)} \kappa_{10} \kappa_2 + \frac{108(2n-3)}{n^2(n-1)^2} \kappa_9 \kappa_3 + 27 \frac{17n^2-49n+35}{n^2(n-1)^3} \kappa_8 \kappa_4 \\
& + 108 \frac{7n^2-20n+16}{n^2(n-1)^3} \kappa_7 \kappa_5 + 27 \frac{17n^2-47n+39}{n^2(n-1)^3} \kappa_6^2 + 27 \frac{37n-70}{n(n-1)^2(n-2)} \kappa_8 \kappa_2^2 \\
& + 324 \frac{19n^2-67n+54}{n(n-1)^3(n-2)} \kappa_7 \kappa_3 \kappa_2 + 162 \frac{65n^2-245n+234}{n(n-1)^2(n-2)} \kappa_6 \kappa_4 \kappa_2 \\
& + 108 \frac{82n^3-481n^2+958-640}{n(n-1)^3(n-2)^2} \kappa_6 \kappa_3^2 + 108 \frac{59n^2-220n+224}{n(n-1)^3(n-2)} \kappa_5^2 \kappa_2 \\
& + 324 \frac{75n^3-473n^2+1016n-756}{n(n-1)^3(n-2)^2} \kappa_5 \kappa_4 \kappa_3 \\
& + 27 \frac{173n^4-1503n^3+4962n^2-7380n+4200}{n(n-1)^3(n-2)^3} \kappa_4^3 \\
& + 108 \frac{71n^2-263n+234}{(n-1)^3(n-2)^2} \kappa_6 \kappa_2^3 + 648 \frac{79n^2-343n+378}{(n-1)^3(n-2)^2} \kappa_5 \kappa_8 \kappa_2^2 \\
& + 486 \frac{63n^2-290n+352}{(n-1)^3(n-2)^3} \kappa_4^2 \kappa_2^2 + 972 \frac{99n^3-688n^2+1612n-1280}{(n-1)^3(n-2)^3} \kappa_4 \kappa_3^2 \kappa_2 \\
& + 162 \frac{87n^3-594n^2+1420n-1176}{(n-1)^3(n-3)^3} \kappa_3^4 + 972 \frac{29n^2-121n+118}{(n-1)^3(n-2)^3} \kappa_4 \kappa_2^4 \\
& + 648n \frac{103n^2-510n+640}{(n-1)^3(n-2)^3} \kappa_5^2 \kappa_3^2 + 648n^2 \frac{5n-12}{(n-1)^3(n-2)^3} \kappa_2^6, \tag{62}
\end{aligned}$$

$$\begin{aligned}
\kappa(2^6) = & \frac{1}{n^5} \kappa_{12} + \frac{60}{n^4(n-1)} \kappa_{10} \kappa_2 + \frac{160(n-2)}{n^4(n-1)^2} \kappa_9 \kappa_3 + 240 \frac{2n^2-5n+4}{n^4(n-1)^3} \kappa_8 \kappa_4 \\
& + 96(n-2) \frac{7n^2-14n+9}{n^4(n-1)^4} \kappa_7 \kappa_5 + 4 \frac{113n^4-520n^3+950n^2-800n+265}{n^4(n-1)^5} \kappa_6^2 \\
& + \frac{1200}{n^8(n-1)^2} \kappa_8 \kappa_2^2 + 4800 \frac{n-2}{n^8(n-1)^3} \kappa_7 \kappa_3 \kappa_2 + 2400 \frac{5n^2-12n+9}{n^8(n-1)^4} \kappa_6 \kappa_4 \kappa_2 \\
& + 160(n-2) \frac{31n-53}{n^8(n-1)^4} \kappa_6 \kappa_3^2 + 960(n-2) \frac{6n^2-12n+7}{n^8(n-1)^5} \kappa_5^2 \kappa_2 \\
& + 1920(n-2) \frac{9n^2-23n+16}{n^8(n-1)^5} \kappa_6 \kappa_4 \kappa_3 + 480 \frac{11n^3-41n^2+59n-31}{n^8(n-1)^5} \kappa_4^3 \\
& + \frac{9600}{n^2(n-1)^3} \kappa_6 \kappa_2^3 + \frac{38400(n-2)}{n^8(n-1)^4} \kappa_6 \kappa_3 \kappa_2^2 + 9600 \frac{4n^2-9n+6}{n^2(n-1)^5} \kappa_4^2 \kappa_2^2 \\
& + 28800 \frac{2n^2-7n+6}{n^2(n-1)^5} \kappa_4 \kappa_3^2 \kappa_2 + 960(n-2) \frac{5n-12}{n^2(n-1)^5} \kappa_3^4 + \frac{28800}{n(n-1)^4} \kappa_4 \kappa_2^4 \\
& + 88400 \frac{n-2}{n(n-1)^5} \kappa_3^2 \kappa_2^3 + \frac{3840}{(n-1)^5} \kappa_2^6. \tag{65}
\end{aligned}$$

Some idea of the advantage of using the cumulative moment functions in place of the moments will be obtained by comparing the above formula (14) with the corresponding formula as obtained by Tchouproff, and corrected by Church :

$$\begin{aligned}
 {}_2M_4 = & \frac{3}{n^2} (\mu_4 - \mu_2'^2) + \frac{1}{n^3} (\mu_8 - 4\mu_6\mu_2 - 24\mu_5\mu_3 - 15\mu_4^2 \\
 & + 48\mu_4\mu_2^2 + 96\mu_3^2\mu_2 - 30\mu_2^4) \\
 & - \frac{1}{n^4} (4\mu_8 - 40\mu_6\mu_2 - 96\mu_5\mu_3 - 54\mu_4^2 + 336\mu_4\mu_2^2 + 528\mu_3^2\mu_2 - 306\mu_2^4) \\
 & - \frac{1}{n^5} (6\mu_8 - 96\mu_6\mu_2 - 176\mu_5\mu_3 - 102\mu_4^2 + 924\mu_4\mu_2^2 + 1232\mu_3^2\mu_2 - 1044\mu_2^4) \\
 & - \frac{1}{n^6} (4\mu_8 - 88\mu_6\mu_2 - 160\mu_5\mu_3 - 95\mu_4^2 + 1050\mu_4\mu_2^2 + 1360\mu_3^2\mu_2 - 1395\mu_2^4) \\
 & + \frac{1}{n^7} (\mu_8 - 28\mu_6\mu_2 - 56\mu_5\mu_3 - 35\mu_4^2 + 420\mu_4\mu_2^2 + 560\mu_3^2\mu_2 - 630\mu_2^4).
 \end{aligned}$$

The term involving  $\kappa_2$  only in  $\kappa(2^n)$  is already known as the  $r$ -th semi-invariant of the distribution of the variance for samples from the normal curve, and is simply  $2^{r-1} \cdot (r-1)! / (n-1)^{r-1}$ . The corresponding term in  $\kappa(3^4)$  is of interest as showing that the distribution of  $k_3$  in samples from a normal distribution, though necessarily symmetrical, yet tends somewhat slowly to normality. Comparing the first term of (17) with that of (4) it is evident that

$$\frac{\kappa(3^4)}{\{\kappa(3^2)\}^2} = \frac{18(5n-12)}{(n-1)(n-2)},$$

or is somewhat greater than  $90/n$ , a fact which indicates that the occurrence of values of  $k_3$  greater than 2 or 3 times its standard error will, except in very large samples, be materially more frequent than one would judge from an assumed normal distribution. The effect upon tests of normality will be examined in §§ 11 and 12.

### 8. Bivariate and multivariate distributions.

The extension to bivariate and multivariate data of the methods of classification and calculation developed above is of both practical and theoretical importance. Apart from the variance the product moment of a bivariate distribution is the most important of all moment statistics.

Moreover, the multivariate formulae, by reason of their greater number, and the confusion caused by the various possible notations in

which they may be expressed, are particularly in need of orderly classification. It will be found, in addition, that the examination of the multivariate formulae in their generality throws much light on the expressions already obtained.

It will be seen that just as the univariate formulae correspond to all the possible partitions of unipartite numbers, so the multivariate formulae correspond to all the possible partitions of multipartite numbers, having multiplicities equal to the number of variates.

To make the notation clear let us consider, in the first place, two variates only, and let the frequency with which the two variates  $x$  and  $y$  fall simultaneously in the ranges  $dx$  and  $dy$  be

$$df = \phi dx dy,$$

in which  $\phi$  is the simultaneous frequency function of  $x$  and  $y$ .

The general moment about any origin is defined as

$$\mu_{pq} = \iint x^p y^q \phi dx dy$$

over the whole range of possible values of the variates. So far as these moments have a meaning we can build up the expression

$$M = \sum_{p=0} \sum_{q=0} \mu_{pq} \frac{t_1^p}{p!} \frac{t_2^q}{q!},$$

and equally, with the same limitation, the coefficients of the expression

$$K = \log M = \sum_{p=0} \sum_{q=0} \kappa_{pq} \frac{t_1^p}{p!} \frac{t_2^q}{q!}$$

will be well defined. The general expressions connecting the cumulative moment functions,  $\kappa$ , with the moments,  $\mu$ , of the simultaneous distribution are analogous to those given for univariate distribution; if

$$\{(p_1 p'_1)^{\pi_1} (p_2 p'_2)^{\pi_2} \dots\}$$

is any partition of the bipartite number  $\tau, s$  consisting of  $\rho$  parts,

$$\kappa_{\tau, s} = S \left\{ \frac{(-)^{\rho-1} (\rho-1)!}{\pi_1! \pi_2! \dots} \frac{r!}{(p_1!)^{\pi_1} (p_2!)^{\pi_2} \dots} \frac{s!}{(p'_1!)^{\pi_1} (p'_2!)^{\pi_2} \dots} \mu_{p_1 p'_1}^{\pi_1} \mu_{p_2 p'_2}^{\pi_2} \dots \right\}$$

and

$$\mu_{\tau, s} = S \left\{ \frac{1}{\pi_1! \pi_2! \dots} \frac{r!}{(p_1!)^{\pi_1} (p_2!)^{\pi_2} \dots} \frac{s!}{(p'_1!)^{\pi_1} (p'_2!)^{\pi_2} \dots} \kappa_{p_1 p'_1}^{\pi_1} \kappa_{p_2 p'_2}^{\pi_2} \dots \right\},$$

the summation being taken over all possible partitions. For any sample, we may define  $s_{pq}$  as the sum of the values of  $x^p y^q$  for each pair of values

in the sample, and obtain, as for single variates, the statistics  $k_{11}$ ,  $k_{21}$ ,  $k_{31}$ ,  $k_{22}$ , etc., as expressions in terms of these sums, with such coefficients that the mean value of  $k_{pq}$  shall be  $\kappa_{pq}$ . Thus we have

$$k_{11} = \frac{1}{n-1} \left( s_{11} - \frac{1}{n} s_{10} s_{01} \right),$$

$$k_{21} = \frac{n}{(n-1)(n-2)} \left( s_{21} - \frac{2}{n} s_{10} s_{11} - \frac{1}{n} s_{20} s_{01} + \frac{2}{n^2} s_{10}^2 s_{01} \right),$$

$$k_{31} = \frac{n}{(n-1)(n-2)(n-3)} \left\{ (n+1) s_{31} - \frac{n+1}{n} s_{30} s_{01} - \frac{3(n-1)}{n} s_{11} s_{20} \right. \\ \left. - \frac{3(n+1)}{n} s_{21} s_{10} + \frac{6}{n} s_{11} s_{10}^2 + \frac{6}{n} s_{20} s_{10} s_{01} - \frac{6}{n^2} s_{01} s_{10}^3 \right\},$$

$$k_{22} = \frac{n}{(n-1)(n-2)(n-3)} \left\{ (n+1) s_{22} - 2 \frac{n+1}{n} s_{21} s_{01} - \frac{n+1}{n} s_{12} s_{10} \right. \\ \left. - \frac{n-1}{n} s_{20} s_{02} - 2 \frac{n-1}{n} s_{11}^2 + \frac{8}{n} s_{11} s_{01} s_{10} \right. \\ \left. + \frac{2}{n} s_{02} s_{10}^2 + \frac{2}{n} s_{20} s_{01}^2 - \frac{6}{n^2} s_{10}^2 s_{01}^2 \right\}.$$

The mean value of any product involving such statistics, as, for example,  $k_{20} k_{11}$ , may be evaluated in terms of the cumulative moment functions of the bivariate distribution; such mean values may be written

$$\overline{k_{20} k_{11}} \equiv \mu \begin{pmatrix} 2 & 1 \\ 0 & 1 \end{pmatrix},$$

giving one line to each variate; its value is easily found to be

$$\frac{1}{n} \kappa_{31} + \frac{n+1}{n-1} \kappa_{20} \kappa_{11}.$$

Hence, subtracting the product of the mean values,  $\kappa_{20} \kappa_{11}$ , we have the formula

$$\kappa \begin{pmatrix} 2 & 1 \\ 0 & 1 \end{pmatrix} = \frac{1}{n} \kappa_{31} + \frac{2}{n-1} \kappa_{20} \kappa_{11} \quad (1a)$$

in which each column represents the particular statistic entering into the product, and the marginal column found by summing each row is the multipartite number (31) representing the degree in which each variate is involved. Similarly, we may deduce the two formulae for partitions

of the bipartite number (22), namely

$$\kappa \begin{pmatrix} 2 & 0 \\ 0 & 2 \end{pmatrix} = \frac{1}{n} \kappa_{22} + \frac{2}{n-1} \kappa_{11}^2 \quad (1b)$$

and 
$$\kappa \begin{pmatrix} 1 & 1 \\ 1 & 1 \end{pmatrix} = \frac{1}{n} \kappa_{22} + \frac{1}{n-1} \kappa_{11}^2 + \frac{1}{n-1} \kappa_{02} \kappa_{20}, \quad (1c)$$

representing the product moment of the estimates of variance of the two correlated variates, and the variance of the estimated product moment.

It will be observed that by equating the two variates, which is carried out by summing the columns of the partition, and replacing the two suffixes of each  $\kappa$  by their sum, equations (1a), (1b), and (1c) are reduced to equation (1). As with univariate formulae, the partitions involving parts of the first degree may be directly derived from formulae of lower degree and therefore need receive no separate consideration.

With more than two variates the bivariate notation may be extended to the use of three or more rows in the representation of a partition of a tripartite number, and three or more suffixes to the parameters  $\kappa$ . The remaining formulae of the fourth degree are therefore

$$\kappa \begin{pmatrix} 2 & 0 \\ 0 & 1 \\ 0 & 1 \end{pmatrix} = \frac{1}{n} \kappa_{211} + \frac{2}{n-1} \kappa_{110} \kappa_{101}, \quad (1d)$$

$$\kappa \begin{pmatrix} 1 & 1 \\ 1 & 0 \\ 0 & 1 \end{pmatrix} = \frac{1}{n} \kappa_{211} + \frac{1}{n-1} \kappa_{200} \kappa_{011} + \frac{1}{n-1} \kappa_{101} \kappa_{110}, \quad (1e)$$

$$\kappa \begin{pmatrix} 1 & 0 \\ 1 & 0 \\ 0 & 1 \\ 0 & 1 \end{pmatrix} = \frac{1}{n} \kappa_{1111} + \frac{1}{n-1} \kappa_{1010} \kappa_{0101} + \frac{1}{n-1} \kappa_{1001} \kappa_{0110}, \quad (1*)$$

representing the partitions of the tripartite number (211) and of the quadripartite (1111), ignoring such as have unitary parts.

Just as equation (1) may be derived from either of equations (1a), (1b), or (1c) by identifying the variates, so, by equating appropriate variates, (1a) may be derived from (1d), or (1b) from (1d), or (1c) from (1e), and finally all can be derived from the general multivariate formula (1\*).

It appears, therefore, that the formulae appropriate for both univariate and multivariate distributions may all be expressed in terms of those representing partitions of the multipartite number (1<sup>n</sup>). Thus of the

sixth degree, a series of formulae, of which formula (3) is the final condensation, will be given by the partition of the multipartite ( $1^6$ ) into parts ( $1^40^2$ ) and ( $0^41^2$ ), a series of formulae reducing to (4) by the partition into the parts ( $1^30^3$ ) and ( $0^31^3$ ), and a series of formulae reducing to (5) by the partition into parts ( $1^20^4$ ), ( $0^21^20^2$ ) and ( $0^41^2$ ). The presentation of formulae of the type here discussed for the case of many variates might therefore be completed by the tabulation of the general multivariate formulae ( $2^*$ ), ( $3^*$ ), etc.

The disadvantage of such a course is that such general formulae will consist of a large number of terms equal to the sum of the coefficients (of the highest powers of  $n$ ) of the formulae already tabulated, and that each term will consist of a product of  $\kappa$ 's, each having as many suffixes as the degree of the equation. The general formulae are therefore extremely cumbrous, and, as the suffixes will consist merely of repetitions in different orders of the numbers 0 and 1, it will be of more value if general rules can be found by which these particular combinations are to be selected. Such rules will then apply to the univariate and less general multivariate cases, the coefficients being merely the number of ways in which each selection can be made.

Now the suffixes of the product terms are merely other partitions of the same number, whether unipartite or multipartite, of which one particular partition specifies our formula; we are therefore concerned with the difficult question of the relations which can exist between different partitions of the same number. This question may be considered solely with respect to unipartite numbers, for if the rules can be made out which govern the coefficients in such cases, the same identical rules must apply to multipartite numbers by reason of the methods by which one formula may be condensed into another. For example, if we start with the partition ( $2^2$ ) of the number 4, in conjunction with the rule that only such partitions are to be considered as in each part involve elements from both parts of the old partition, we should obtain equally the coefficient 2 of the term  $\kappa_2^2$ , and by applying the same rule to the partition of the multipartite number ( $1^4$ ) into parts ( $1^20^2$ ) and ( $0^21^2$ ) should obtain the terms  $\kappa_{1010}$ ,  $\kappa_{0101}$ , and  $\kappa_{1001}$ ,  $\kappa_{0110}$ , having in both cases the same divisor  $n-1$ .

### 9. Empirical statement of the rules for the direct evaluation of the coefficients.

Although the rules of the combinatorial procedure were not completed before the development of the method of Section 10, yet so much

can be learned by an empirical study of the formulae that it is convenient to make a complete statement of the rules in an empirical form, prior to the demonstration of their validity.

(1) The coefficient of  $\kappa_{q_1}^{x_1} \kappa_{q_2}^{x_2} \dots$  in the expression for  $\kappa(p_1^{r_1} p_2^{r_2} \dots)$  depends on the possible partitions of the second order of which the column totals give the partition  $(p_1^{r_1} p_2^{r_2} \dots)$ , and the row totals give the partition  $(q_1^{x_1} q_2^{x_2} \dots)$ .

For example, the coefficient of  $\kappa_6 \kappa_2^2$  in the expression for  $\kappa(4^2 2)$  may be obtained by inspection of the partitions of the second order

2 2 2	6	2 3 1	6	3 3 .	6
1 1 .	2	1 1 .	2	1 . 1	2
1 1 .	2	1 . 1	2	. 1 1	2
4 4 2	10	4 4 2	10	4 4 2	10

in each of which the sums of the rows constitute the partition  $(62^2)$ , while the sums of the columns constitute the partition  $(4^2 2)$ .

(2) The numerical factor in the contribution made by any partition of the second order is the number of ways in which the totals in the lower margin may be allocated to form a partition of the type considered. The numerical factors corresponding to the three partitions set out above are 72, 192, and 32 respectively. In the first case, for example, the number may be arrived at from the consideration that the pair of units to be separated in the first four may be chosen in six ways, and that these may be assigned partners from the second pair in twelve ways. In the second case we may choose either of the two fours to be parted into  $(21^2)$ , as in the first column, and, whichever is chosen, we may allocate the units in the three columns in twelve, four, and two ways respectively; while, in the third case, we may choose the units from the two fours in sixteen ways and associate them in two ways with the units of the two.

(3) Before considering the general rule for determining the function of  $n$  by which the numerical factor is to be multiplied, it is convenient to note that certain partitions of the second order make no contribution whatever to the coefficient, and so may be neglected at once. The most useful class consists of those in which any row has only one entry other than zero; for example, such partitions as

2 3 1	6
. 1 1	2
2 . .	2
4 4 2	10

are to be ignored. It is obvious for statistical reasons, as has been mentioned above, that  $\kappa_1$  cannot appear in any of these formulae, and as it will be seen that the function of  $n$  involved depends only upon the configuration of the zeros of the partition of the second order, the necessity for this rule will become apparent. More generally, we may exclude any partition in which any set of rows is connected to its complementary set by a single column only.

(4) The usefulness of rule (3) for excluding superfluous partitions is extended by employing it in conjunction with the rule which holds when any column has only one entry other than zero; for in these cases we may introduce the factor  $n^{-1}$  and ignore the column concerned. For example, the partition pattern

$$\begin{array}{ccc} \times & \times & \times \\ \times & \times & . \\ \times & \times & . \end{array}$$

irrespective of its numerical coefficient, is associated with a function of  $n$  which is one  $n$ -th of that associated with

$$\begin{array}{cc} \times & \times \\ \times & \times \\ \times & \times \end{array}$$

Moreover, such a partition as

$$\begin{array}{ccc|c} 4 & 2 & . & 6 \\ . & 1 & 1 & 2 \\ . & 1 & 1 & 2 \\ \hline 4 & 4 & 2 & 10 \end{array}$$

is to be ignored (although every row has two entries) by reason of its connection with

$$\begin{array}{cc} \times & . \\ \times & \times \\ \times & \times \end{array}$$

in which this condition is not fulfilled.

With these criteria of rejection one may easily assure oneself that the three partitions set out above are the only ones which need be considered in that case.

(5) To find, in general, the function of  $n$  with which any pattern is associated, we consider all the possible ways in which the rows can be

separated into 1, 2, 3, ... separate groups, or separates. Thus with three rows we have one separation into one separate, with which is associated the factor  $n$ ; three separations into two separates, with which is associated the factor  $n(n-1)$ ; and one separation into three separates, with which is associated the factor  $n(n-1)(n-2)$ . In each of these five separations we count in how many separates each column is represented by entries other than zero. If in one separate, that column contributes a factor  $n^{-1}$ ; if in 2, 3, 4, ... separates, the factors are

$$\begin{array}{ccc} -1 & 2! & -3! \\ n(n-1)' & n(n-1)(n-2)' & n(n-1)(n-2)(n-3)' \end{array}$$

In applying this rule all patterns which are resolvable into two parts, each confined to separable sets of rows and columns, must be ignored.

As an example, consider the five possible separations of the pattern

$$\begin{array}{c} \times \times \\ \times \times \\ \times \times ; \end{array}$$

the first supplies the term

$$\frac{n}{n^2} = \frac{1}{n},$$

the separations into two separates supply

$$\frac{3n(n-1)}{n^2(n-1)^2} = \frac{3}{n(n-1)},$$

while the separation into three separates gives

$$\frac{4n(n-1)(n-2)}{n^2(n-1)^2(n-2)^2} = \frac{4}{n(n-1)(n-2)},$$

the total being  $n/\{(n-1)(n-2)\}$ , the function appropriate to this pattern.

It is equally easy to verify that the functions appropriate to the patterns

$$\begin{array}{cc} \times \times \times & \times \times . \\ \times \times . & \times . \times \\ \times . \times & . \times \times \end{array}$$

both reduce to  $1/(n-1)^2$ . The required coefficient is therefore

$$\frac{72}{(n-1)(n-2)} + \frac{224}{(n-1)^2} = \frac{8(37n-65)}{(n-1)^2(n-1)},$$

as appears in formula 28.

It will be obvious from the preceding section that the same rules must be applicable to multivariate problems, the only difference being that the column totals are then regarded as consisting of objects of two or more kinds. For example, to find the coefficient of  $\kappa_{33}\kappa_{11}^2$  in the expression for  $\kappa \begin{pmatrix} 2 & 2 & 1 \\ 2 & 2 & 1 \end{pmatrix}$ , it is merely necessary to note that the second order partitions of the bipartite (55) corresponding to the three partitions of 10, used above, can be allocated in 20, 48, and 8 ways respectively, yielding a coefficient

$$\frac{4(19n-33)}{(n-1)^2(n-2)}.$$

Alternatively the contributions to the coefficient of the univariate formula may be each split up among the six coefficients by which it is replaced in the bivariate formula, giving in this case

$$4(19n-33)\kappa_{33}\kappa_{11}^2 + 8(11n-20)\kappa_{33}\kappa_{20}\kappa_{02} + 8(7n-12)\kappa_{42}\kappa_{11}\kappa_{02} \\ + 8(7n-12)\kappa_{24}\kappa_{11}\kappa_{20} + 2(5n-9)\kappa_{81}\kappa_{20}^2 + 2(5n-9)\kappa_{15}\kappa_2^2$$

in place of

$$8(37n-65)\kappa_8\kappa_2^2.$$

In the same way the appropriate subdivision of the other bivariate and multivariate formulae may be obtained from an examination of the same set of two-way partitions, and it will evidently be sufficient for practical purposes to tabulate all the univariate formulae up to a given degree in order that all the corresponding multivariate formulae should be rapidly obtainable.

The algebraic equivalents of a number of the more commonly occurring patterns are given on pages 223-226.

### *Some useful patterns.*

#### *Two rows.*

$$\begin{array}{lll} \begin{array}{c} \times \times \\ \times \times \end{array} \frac{1}{n-1} & \begin{array}{c} \times \times \times \\ \times \times \times \end{array} \frac{n-2}{n(n-1)^2} & \begin{array}{c} \times \times \times \times \\ \times \times \times \times \end{array} \frac{n^2-3n+3}{n^3(n-1)^3} \end{array}$$

In general, if  $\alpha = -\{1/(n-1)\}$ , we have  $1/(n^{p-1})(1-\alpha^{p-1})$ .

Three rows.

$$\begin{array}{ccc} \begin{array}{c} \times \times \\ \times \times \\ \times \times \end{array} \frac{n}{(n-1)(n-2)} & \begin{array}{c} \times \times \times \\ \times \times \times \\ \times \times \times \end{array} \frac{n^2-6n+10}{(n-1)^2(n-2)^2} & \begin{array}{c} \times \times \times \\ \times \times \times \\ \times \times \end{array} \frac{n-3}{(n-1)^2(n-2)} \end{array}$$

$$\begin{array}{ccc} \begin{array}{c} \times \times \times \\ \times \cdot \times \equiv \times \cdot \times \\ \times \times \cdot \quad \times \times \cdot \end{array} \frac{1}{(n-1)^2} & \begin{array}{c} \times \times \times \times \\ \times \times \times \times \\ \times \times \times \times \end{array} \frac{n^4-9n^3+33n^2-60n+48}{n(n-1)^3(n-2)^3} \end{array}$$

$$\begin{array}{ccc} \begin{array}{c} \times \times \times \times \\ \times \times \times \times \\ \times \times \times \cdot \end{array} \frac{(n-3)(n^2-4n+6)}{n(n-1)^3(n-2)^3} & \begin{array}{c} \times \times \times \times \\ \times \times \times \times \\ \times \times \cdot \cdot \end{array} \frac{n^2-4n+5}{n(n-1)^3(n-2)} \end{array}$$

$$\begin{array}{ccc} \begin{array}{c} \times \times \times \times \\ \times \times \cdot \times \\ \times \times \times \cdot \end{array} \frac{n^2-5n+7}{n(n-1)^3(n-2)} & \begin{array}{c} \times \cdot \times \times \\ \times \times \cdot \times \\ \times \times \times \cdot \end{array} \frac{n-3}{n(n-1)^3} \end{array}$$

$$\begin{array}{ccc} \begin{array}{c} \times \times \times \times \\ \times \cdot \times \times \equiv \times \cdot \times \times \\ \times \times \cdot \cdot \quad \times \times \cdot \cdot \end{array} \frac{n-2}{n(n-1)^3} & \begin{array}{c} \times \times \times \times \\ \cdot \cdot \times \times \\ \times \times \cdot \cdot \end{array} \frac{1}{n(n-1)^2} \end{array}$$

Four rows.

$$\begin{array}{ccc} \begin{array}{c} \times \times \\ \times \times \\ \times \times \\ \times \times \end{array} \frac{n(n+1)}{(n-1)(n-2)(n-3)} & \begin{array}{c} \times \times \times \\ \times \times \times \\ \times \times \times \\ \times \times \times \end{array} \frac{n^4-12n^3+51n^2-74n-18}{(n-1)^2(n-2)^2(n-3)^2} \end{array}$$

$$\begin{array}{ccc} \begin{array}{c} \times \times \times \\ \times \times \times \\ \times \times \times \\ \times \times \cdot \end{array} \frac{n^3-8n^2+17n+2}{(n-1)^2(n-2)^2(n-3)} & \begin{array}{c} \times \times \times \\ \times \times \times \\ \times \times \cdot \\ \times \times \cdot \end{array} \frac{n^2-4n-1}{(n-1)^2(n-2)(n-3)} \end{array}$$

$$\begin{array}{ccc} \begin{array}{c} \times \times \times \\ \times \times \times \\ \times \cdot \times \\ \times \times \cdot \end{array} \frac{n(n-4)}{(n-1)^2(n-2)^2} & \begin{array}{c} \times \times \times \\ \cdot \times \times \\ \times \cdot \times \\ \times \times \cdot \end{array} \frac{n(n-3)}{(n-1)^2(n-2)^2} \end{array}$$

$$\begin{array}{ccc} \begin{array}{c} \times \times \times \\ \times \cdot \times \equiv \times \cdot \times \\ \times \times \cdot \quad \times \times \cdot \\ \times \times \cdot \quad \times \times \cdot \end{array} \frac{n}{(n-1)^2(n-2)} \end{array}$$

$$\begin{array}{ccc} \begin{array}{c} \times \times \cdot \times \\ \times \times \cdot \times \\ \times \times \times \cdot \\ \times \times \times \cdot \end{array} \begin{array}{c} \times \times \times \times \\ \times \times \cdot \times \\ \times \times \cdot \cdot \\ \times \times \times \cdot \end{array} \frac{n^3-7n^2+13n+1}{n(n-1)^3(n-2)(n-3)} \end{array}$$

$$\begin{array}{cc}
 \begin{array}{c} \times \times \times \times \\ \times \times \times \times \\ \times \times . . \\ \times \times . . \end{array} & \frac{n^3 - 5n^2 + 7n + 1}{n(n-1)^3(n-2)(n-3)} \\
 \begin{array}{c} \times \times \times \times \\ . \times \times \times \\ \times . \times \times \\ \times \times . . \end{array} & \frac{n^3 - 8n^2 + 23n - 24}{(n-1)^3(n-2)^3}
 \end{array}$$

$$\begin{array}{cc}
 \begin{array}{c} . \times \times \times \\ \times . \times \times \\ \times \times . \times \\ \times \times \times . \end{array} & \frac{n^3 - 9n^2 + 29n - 32}{(n-1)^3(n-2)^3} \\
 \begin{array}{c} \times . \times \times \\ \times \times . \times \\ \times \times \times . \\ \times \times \times . \end{array} & \frac{n^2 - 7n + 14}{(n-1)^3(n-2)^3}
 \end{array}$$

$$\begin{array}{ccc}
 \begin{array}{c} \times \times \times \times \\ \times . \times \times \\ \times \times . . \\ \times \times \times . \end{array} & \equiv & \begin{array}{c} \times \times \times \times \\ \times . . \times \\ \times \times \times . \\ \times \times \times . \end{array} \equiv \begin{array}{c} . \times \times \times \\ \times \times . \times \\ \times \times \times . \\ \times \times \times . \end{array} \frac{n^2 - 6n + 10}{(n-1)^3(n-2)^2}
 \end{array}$$

$$\begin{array}{cc}
 \begin{array}{c} \times \times \times \times \\ \times \times \times \times \\ . . \times \times \\ \times \times . . \end{array} & \begin{array}{c} \times \times \times \times \\ \times \times \times \times \\ \times . . \times \\ \times \times . . \end{array} \frac{n^2 - 5n + 8}{(n-1)^3(n-2)^2} \\
 \begin{array}{c} . \times \times \times \\ \times . \times \times \\ \times \times . \times \\ \times \times . . \end{array} & \begin{array}{c} \times \times \times \times \\ . \times \times \times \\ \times . \times . \\ \times \times . . \end{array} \frac{n^3 - 5n + 7}{(n-1)^3(n-2)^3}
 \end{array}$$

$$\begin{array}{cc}
 \begin{array}{c} . \times \times \times \\ \times . \times \times \\ \times \times . \times \\ \times \times . . \end{array} & \frac{(n-3)^2}{(n-1)^3(n-2)^2} \\
 \begin{array}{c} \times . \times \times \\ \times \times . \times \\ \times \times . . \\ \times \times \times . \end{array} & \frac{n-4}{(n-1)^3(n-2)}
 \end{array}$$

$$\begin{array}{cccc}
 \begin{array}{c} \times . . \times \\ \times \times . \times \\ \times \times \times . \\ \times \times \times . \end{array} & \begin{array}{c} \times \times \times \times \\ \times . . \times \\ \times \times . . \\ \times \times \times . \end{array} & \begin{array}{c} \times \times \times \times \\ . . \times \times \\ \times \times . \times \\ \times \times . . \end{array} & \begin{array}{c} . \times \times \times \\ . \times \times \times \\ \times . . \times \\ \times \times . . \end{array}
 \end{array}$$

$$\begin{array}{ccc}
 \begin{array}{c} . \times \times \times \\ \times . . \times \\ \times \times . . \\ \times \times \times . \end{array} & \equiv & \begin{array}{c} . . \times \times \\ \times \times . \times \\ \times \times \times . \\ \times \times . . \end{array} \frac{n-3}{(n-1)^3(n-2)}
 \end{array}$$

$$\begin{array}{c}
 \times \times \times \times \\
 \times \times . . \\
 \times \times . . \\
 . . \times \times
 \end{array}
 \frac{1}{(n-1)^3(n-2)}$$

$$\begin{array}{cccccc}
 \begin{array}{c} \times \times \times \times \\ \times . . \times \\ \times \times . . \\ \times . \times . \end{array} & \begin{array}{c} \times \times \times \times \\ . . \times \times \\ \times . . \times \\ \times \times . . \end{array} & \begin{array}{c} . \times \times \times \\ . . \times \times \\ \times . . \times \\ \times \times . . \end{array} & \begin{array}{c} . \times \times . \\ . \times \times \times \\ \times . . \times \\ \times \times . . \end{array} & \begin{array}{c} . \times \times \times \\ \times . \times \times \\ \times \times . . \\ \times \times . . \end{array}
 \end{array}$$

$$\begin{array}{ccc}
 \begin{array}{c} . \times \times \times \\ \times \times . . \\ \times . \times . \\ \times . . \times \end{array} & \equiv & \begin{array}{c} . . \times \times \\ \times \times . . \\ \times \times . . \\ \times \times \times . \end{array} \frac{1}{(n-1)^3}
 \end{array}$$

*Five and six row patterns.*

$$\begin{array}{ccc}
 \times \times . & \times \times . & \times \times . \quad \times \times . \\
 \times \times . & \times \times . & \times \times . \quad \times \times . \\
 \times . \times \equiv \times . \times & \frac{n^2}{(n-1)^3(n-2)^2} & \times \times . \equiv \times \times . \quad \frac{n(n+1)}{(n-1)^2(n-2)(n-3)} \\
 \times . \times & \times . \times & \times . \times \quad \times . \times \\
 . \times \times & \times \times \times & . \times \times \quad \times \times \times
 \end{array}$$

$$\begin{array}{ccc}
 \times \times & & \times \times \times \\
 \times \times & & \times \times . \\
 \times \times & \frac{n^2(n+5)}{(n-1)(n-2)(n-3)(n-4)} & \times \times . \quad \frac{n(n^2-4n-1)}{(n-1)^2(n-2)^2(n-3)} \\
 \times \times & & \times . \times \\
 \times \times & & . \times \times
 \end{array}$$

$$\begin{array}{ccc}
 \times \times \times & & \times \times \times \\
 \times \times \times & & \times \times \times \\
 \times \times . & \frac{n(n^2-5n-2)}{(n-1)^2(n-2)^2(n-3)} & \times \times . \quad \frac{n(n^2-4n-9)}{(n-1)^2(n-2)(n-3)(n-4)} \\
 \times \times . & & \times \times . \\
 \times . \times & & \times \times .
 \end{array}$$

$$\begin{array}{ccc}
 \times \times \times & & \times \times . \\
 \times \times \times & & \times \times . \\
 \times \times . & \frac{n(n^3-9n^2+19n+5)}{(n-1)^2(n-2)^2(n-3)^2} & \times . \times \quad \frac{n(n+1)(n^2-5n+2)}{(n-1)^2(n-2)^2(n-3)^2} \\
 \times . \times & & . \times \times \\
 . \times \times & & . \times \times
 \end{array}$$

$$\begin{array}{ccc}
 \times \times . & & \times \times \\
 \times \times . & & \times \times \\
 \times \times . & \frac{n^2(n+1)}{(n-1)^2(n-2)^2(n-3)} & \times \times \quad \frac{n(n+1)(n^2+15n-4)}{(n-1)(n-2)(n-3)(n-4)(n-5)} \\
 \times . \times & & \times \times \\
 . \times \times & & \times \times
 \end{array}$$

The general formula for the two-column pattern with  $r$  rows is easily found, by enumerating the separations into 1, 2, 3, ... separates, to be

$$\Delta^p(0^r) = \sum_{p=1}^r p \quad n(n-1) \dots (n-p+1),$$

where  $\Delta^p(0^r)$  stands for the leading  $p$ -th advancing difference of the series  $0^r, 1^r, 2^r, \dots$

#### 10. *Demonstration of the combinatorial method.*

To demonstrate the validity of the rules which have been stated, it is useful to consider in what manner the generating function  $M$  will

be modified by a functional transformation of the variates. In the case of a single variate  $x$  we have the function

$$M = 1 + \mu_1 \kappa + \mu_2 \frac{\kappa^2}{2!} + \dots,$$

the coefficients of which give the mean value of all powers of  $x$  in the population. By what operation should the function  $M$  be transformed so as to give the corresponding function appropriate to a new variate  $\xi$ , which is a known function of  $x$ ? Suppose that

$$\xi = f(x) = c_0 + c_1 x + c_2 x^2 + \dots,$$

then the mean value of  $\xi$  is

$$\mu'_1 = c_0 + c_1 \mu_1 + c_2 \mu_2 + \dots,$$

which may be written

$$c_0 + c_1 \frac{d}{dt} M + c_2 \frac{d^2}{dt^2} M + \dots,$$

or

$$f\left(\frac{d}{dt}\right) M,$$

where  $t$  is made to vanish after operation.

Moreover, the mean value of the  $r$ -th power of  $\xi$  will be given, at least formally, by the equation

$$\mu'_r = \left\{ f\left(\frac{d}{dt}\right) \right\}^r M,$$

and the new generating function,

$$M' = 1 + \mu'_1 \tau + \mu'_2 \frac{\tau^2}{2!} + \dots,$$

may be written

$$e^{\tau f} M,$$

in which the operator is supposed to be expanded in powers of  $d/dt$  before attacking the operand.

The corresponding relationship for simultaneous variation is easily found. In such cases  $M$  will be a function of two or more variables  $t_1, t_2, \dots$  corresponding to the variates  $x, y, \dots$ ; the new variates will be given functions of the old

$$\xi_1 = f_1(x, y, \dots),$$

$$\xi_2 = f_2(x, y, \dots),$$

and the operative expression for the transformation of  $M$  is

$$M' = e^{\tau_1 f_1 + \tau_2 f_2 \dots} M.$$

To apply this result to univariate sampling problems, consider the  $n$  observations of the sample as our  $n$  original variates, and the symmetric functions  $k_1, k_2, \dots$  as the new variates the generating function of which is required. Then, considering first the operand, for the first observation  $x$ , the  $\mu$  generator is  $e^{K(t)}$ , where  $K$  is the  $\kappa$  generator of the population sampled, *i.e.*

$$K(t) = \kappa_1 t + \kappa_2 \frac{t^2}{2!} + \dots$$

Moreover, since the  $n$  observations are independent, their simultaneous  $\kappa$  generator will be merely the sum of the individual generators, so that our operand is

$$\exp \left\{ \kappa_1 s_1 + \kappa_2 \frac{s_2}{2!} + \dots \right\},$$

in which

$$s_r = \sum_{v=1}^n (t_v^r).$$

We may note at once that the coefficient of  $\kappa_{q_1}^{x_1} \kappa_{q_2}^{x_2} \dots$  in the operand is

$$\frac{s_{q_1}^{x_1}}{(q_1!)^{x_1} x_1!} \frac{s_{q_2}^{x_2}}{(q_2!)^{x_2} x_2!} \dots$$

The  $\mu$  generator of the simultaneous distribution of the  $k$  statistics will be given by the operator

$$e^{\tau_1 k_1 + \tau_2 k_2 + \dots + \tau_n k_n},$$

in which  $k_\nu$  is interpreted as the same function of  $d/dt_1, d/dt_2, \dots$  as the corresponding  $k$  statistic is of  $x_1, x_2, \dots, x_n$ . The property by which these statistics were defined, namely that the mean value of  $k_\nu$  should be  $\kappa_\nu$ , is now seen to imply that

$$k_\nu \left( \frac{s_\nu}{\nu!} \right) = 1;$$

but

$$k_\nu \left( \frac{s_{\nu_1}}{\nu_1!} \frac{s_{\nu_2}}{\nu_2!} \dots \right) = 0,$$

where  $(\nu_1, \nu_2, \dots)$  is any partition of  $\nu$ . If, for example, the partition is of two parts,

$$s_{\nu_1} s_{\nu_2} = \sum_1^n (t^{\nu_1 + \nu_2}) + \sum_1^{n(n+1)} (t^{\nu_1} t^{\nu_2}),$$

in which  $t$  and  $t'$  are different members of the set  $t_1, \dots, t_n$ , it follows that  $k_p$  must contain, in addition to the simple term

$$\frac{1}{n} S \left( \frac{d}{dt} \right)^n,$$

terms for all two-part partitions of the form

$$\frac{-1}{n(n-1)} \frac{\nu!}{\nu_1! \nu_2!} S \left\{ \left( \frac{d}{dt_1} \right)^{\nu_1} \left( \frac{d}{dt'} \right)^{\nu_2} \right\}$$

except when  $\nu_1 = \nu_2$ , when each operator finds two terms on which it can act, and its coefficient is therefore to be halved. Thus, if we write

$$g(p_1^{\pi_1} p_2^{\pi_2} \dots) = \frac{(-)^{\rho-1} (\rho-1)!}{n(n-1) \dots (n-\rho+1)} \\ \times S \left\{ \left( \frac{d}{dt_{\nu_1}} \right)^{\nu_1} \dots \left( \frac{d}{dt_{\nu_{\pi_1}}} \right)^{\pi_1} \left( \frac{d}{dt_{\nu_1}} \right)^{\nu_1} \dots \left( \frac{d}{dt_{\nu_{\pi_2}}} \right)^{\pi_2} \dots \right\},$$

where  $\rho = \pi_1 + \pi_2 + \dots$  and  $t_{\nu_1}$ , etc. are any selection of  $\rho$  out of the  $n$  variables  $t$ , the summation being extended over all such selections, then

$$k_p = \Sigma \frac{p!}{(p_1!)^{\pi_1} \pi_1! (p_2!)^{\pi_2} \pi_2! \dots} g(p_1^{\pi_1} p_2^{\pi_2} \dots),$$

the summation being taken over all partitions of  $p$ .

This structure of the  $k$  operator makes it possible to think of the  $p$  acts of differentiation in each operator as  $p$  separate objects, the partitions of which, represented by the  $g$  operators, occur each in as many ways as the objects can be arranged in that partition. We may thus use a two-way partition to assign how many of these operations are effective against each of a series of factors  $s_{q_1} s_{q_2}$  constituting the operand.

Let now this operand product be expanded in a number of terms of the form

$$z(a, b, c) = S(t^a t'^b t''^c),$$

the summation being taken over all the  $n(n-1)(n-2)$  different ways of selecting  $t$ ,  $t'$ , and  $t''$  from among the set  $t_1, \dots, t_n$ . This will then be a  $z$  term for every possible separation of the partition  $(q_1^{x_1} q_2^{x_2} \dots)$  into one or more separates. For each two-way partition chosen all these separations will contribute to the result, and with the same numerical coefficient, apart from that contained in the  $g$  operators, equal to the number of ways of allocating the objects in the two-way partition.

The number of terms in the  $z$  corresponding to any separation into  $a$  separates is  $n(n-1) \dots (n-a+1)$ , and this, combined with the factors in the  $g$  operators, gives the functions of  $n$  corresponding to any two-way partition according to rule (5). There remains, however, in  $M'$  a number of terms corresponding to two-way partitions, in which the columns may be divided into two classes, each confined to different sets of rows. These introduce terms of a higher order in  $n$ , which are obliterated when we find  $K' = \log M'$ , for in these cases the additional term in  $M'$  will be of the form  $AB$ , where both  $A$  and  $B$  occur also as other terms in  $M'$ .

### 11. Measures of departure from normality.

The statistical inefficiency of moment statistics from distributions differing widely from the normal, except when they are of a special type [10], much reduces their practical importance for curve fitting; but since they are fully efficient for the normal distribution, they provide an ideal basis for testing if an observed sample indicates a significant departure from normality in the population sampled. Significant asymmetry should, in the first instance, be shown by an excessive value of  $k_3$ ; but since the variance of the population is usually unknown, but may be estimated from the value of  $k_2$  observed in the sample, the test of significance will usually involve not the distribution of  $k_3$ , the moments of which are given by such formulae as (4) and (20), but the distribution of the ratio  $k_3 k_2^{-3/2}$ . Since, for the normal distribution, the variance of  $k_3$  is given by

$$\kappa(3^2) = \frac{6n}{(n-1)(n-2)} \kappa_2^3,$$

it will be convenient to show how the moments of such a statistic as

$$x = \sqrt{\left(\frac{(n-1)(n-2)}{6n}\right)} k_3 k_2^{-3/2}$$

may be expressed in terms of the general  $\kappa(p_1^{\pi_1} p_2^{\pi_2} \dots)$ , for all distributions, and its particular value obtained for the normal distribution. This may be done by expanding the factor  $k_2^{-3/2}$  in the form

$$\kappa_2^{-3/2} \left(1 + \frac{k_2 - \kappa_2}{\kappa_2}\right)^{-3/2},$$

whereupon, in virtue of the expressions connecting the moments  $\mu$  with the semi-invariants  $\kappa$ , and the mean value of  $x$  being zero, we can at once

write down the following expansion for its variance :

$$\begin{aligned}\mu_2(x) = \kappa_2(x) = \frac{(n-1)(n-2)}{6n\kappa_2^3} & \left\{ \kappa(3^2) - \frac{3}{\kappa_2} \kappa(3^2 2) + \frac{6}{\kappa_2^2} \{ \kappa(3^2) \kappa(2^2) + \kappa(3^2 2^2) \} \right. \\ & \left. - \frac{10}{\kappa_2^3} \{ 3\kappa(3^2 2) \kappa(2^2) + \kappa(3^2) \kappa(2^3) \} + \frac{15}{\kappa_2^4} \{ 3\kappa(3^2) \kappa^2(2^2) \} \right\},\end{aligned}$$

in which, remembering that a  $\kappa$  of  $p$  parts involves  $n^{-(p-1)}$ , terms beyond  $n^{-2}$  have been omitted, as well as the terms of odd degree which vanish for symmetrical distributions.

Similarly we have

$$\begin{aligned}\mu_4(x) = \frac{(n-1)^2(n-2)^2}{36n^3\kappa_2^4} & \left\{ 3\kappa^2(3^2) + \kappa(3^4) - \frac{6}{\kappa_2} \{ 6\kappa(3^2 2) \kappa(3^2) + \kappa(3^4 2) \} \right. \\ & + \frac{21}{\kappa_2^2} \{ 3\kappa^2(3^2) \kappa(2^2) + 6\kappa^2(3^2 2) \\ & \quad \left. + \kappa(3^4) \kappa(2^2) + 6\kappa(3^2 2^2) \kappa(3^2) \} \\ & - \frac{56}{\kappa_2^3} \{ 18\kappa(3^2 2) \kappa(3^2) \kappa(2^2) + 3\kappa^2(3^2) \kappa(2^3) \} \\ & \left. + \frac{126}{\kappa_2^4} \{ 9\kappa^2(3^2) \kappa^2(2^2) \} \right\}\end{aligned}$$

and

$$\begin{aligned}\mu_6(x) = \frac{(n-1)^3(n-2)^3}{216n^3\kappa_2^5} & \left\{ 15\kappa^3(3^2) + 5\kappa(3^4) \kappa(3^2) + \kappa(3^6) \right. \\ & - \frac{9}{\kappa_2} \{ 45\kappa(3^2 2) \kappa^2(3^2) + 15\kappa(3^4 2) \kappa(3^2) + 15\kappa(3^4) \kappa(3^2 2) \} \\ & + \frac{45}{\kappa_2^2} \{ 15\kappa^3(3^2) \kappa(2^2) + 90\kappa^2(3^2 2) \kappa(3^2) + 45\kappa(3^2 2^2) \kappa^2(3^2) \\ & \quad \left. + 15\kappa(3^4) \kappa(3^2) \kappa(2^2) \} \\ & - \frac{165}{\kappa_2^3} \{ 15\kappa^3(3^2) \kappa(2^3) + 135\kappa(3^2 2) \kappa^2(3^2) \kappa(2^2) \right. \\ & \left. + \frac{495}{\kappa_2^4} \{ 45\kappa^3(3^2) \kappa^2(2^2) \} \right\}.\end{aligned}$$

From these moments of the distribution of  $x$ , the semi-invariants  $\kappa_4(x)$  and  $\kappa_6(x)$  may be obtained by means of the relations

$$\mu_4(x) = \kappa_4(x) + 3\kappa_2^2(x),$$

$$\mu_6(x) = \kappa_6(x) + 15\kappa_4(x) \kappa_2(x) + 15\kappa_2^3(x),$$

giving

$$\begin{aligned}\kappa_4(x) = \frac{(n-1)^2(n-2)^2}{86n^2\kappa_2^6} \bigg\{ & \kappa(3^4) - \frac{18}{\kappa_2} \kappa(3^2 2) \kappa(3^2) + \frac{27}{\kappa_2^2} \kappa^2(3^2) \kappa(2^2) \\ & - \frac{6}{\kappa_2} (3^4 2) + \frac{99}{\kappa_2^2} \kappa^2(3^2 2) + \frac{21}{\kappa_2^2} \kappa(3^4) \kappa(2^2) \\ & + \frac{90}{\kappa_2^2} \kappa(3^2 2^2) \kappa(3^2) - \frac{720}{\kappa_2^2} \kappa(3^2 2) \kappa(3^2) \kappa(2^2) \\ & - \frac{108}{\kappa_2^2} \kappa^2(3^2) \kappa(2^3) + \frac{756}{\kappa_2^4} \kappa^2(3^2) \kappa^2(2^2) \bigg\}\end{aligned}$$

and

$$\begin{aligned}\kappa_6(x) = \frac{(n-1)^3(n-2)^3}{216n^3\kappa_2^9} \bigg\{ & \kappa(3^6) - \frac{45}{\kappa_2} \kappa(3^4 2) \kappa(3^2) - \frac{90}{\kappa_2} \kappa(3^4) \kappa(3^2 2) \\ & + \frac{1350}{\kappa_2^2} \kappa^2(3^2 2) \kappa(3^2) + \frac{405}{\kappa_2^2} \kappa(3^2 2^2) \kappa^2(3^2) \\ & + \frac{270}{\kappa_2^2} \kappa(3^4) \kappa(3^2) \kappa(2^2) - \frac{405}{\kappa_2^3} \kappa^3(3^2) \kappa(2^3) \\ & - \frac{5670}{\kappa_2^3} \kappa(3^2 2) \kappa^2(3^2) \kappa(2^2) + \frac{4860}{\kappa_2^4} \kappa^2(3^2) \kappa^2(2^2) \bigg\},\end{aligned}$$

while no higher semi-invariants contain terms involving only  $n^{-2}$ .

The formulae tabulated give all the values required for  $\kappa_2(x)$ ; thus for samples from the normal distribution

$$\kappa(2^2) = \frac{2}{n-1} \kappa_2^2, \quad \kappa(3^2) = \frac{6n}{(n-1)(n-2)} \kappa_2^3, \quad \kappa(2^3) = \frac{8}{(n-1)^2} \kappa_2^3,$$

$$\kappa(3^2 2) = \frac{6}{n-1} \kappa_2 \kappa(3^2), \quad \kappa(3^2 2^2) = \frac{48}{(n-1)^2} \kappa_2^2 \kappa(3^2),$$

and substituting these values, we find

$$\kappa_2(x) = 1 - \frac{6}{n} + \frac{22}{n^2}.$$

To evaluate  $\kappa_4(x)$  we need in addition

$$\kappa(3^4) = \frac{648(5n-12)n^2}{(n-1)^3(n-2)^3} \kappa_2^6,$$

and the leading term in  $\kappa(3^4 2)$ ; this latter only requires the enumeration of the number of ways of building up two-way partitions of  $(3^4 2)$  with row totals  $(2^7)$ , or the number of ways of connecting up the symbolical

figures

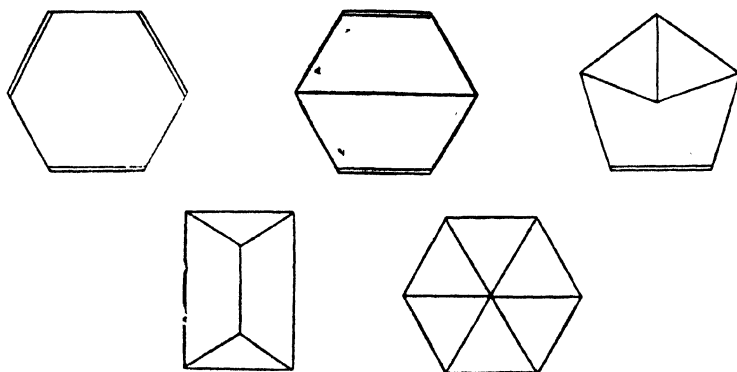


which can be done in 15548, 7774 and 15548 ways respectively, showing that  $\kappa(3^4 2)$  from normal samples is approximately  $38880n^{-4}$ .

With this value, that of  $\kappa_4(x)$  is evaluated as

$$\kappa_4(x) = \frac{36}{n} - \frac{1296}{n^2}.$$

Finally, for  $\kappa_6(x)$  the only new  $\kappa$  required is  $\kappa(3^6)$ , involving the figures having six points from each of which three lines radiate :—



which supply a contribution of  $47520n^{-2}$  to  $\kappa_6(x)$ , or, with the other terms, lead to the value

$$\kappa_6(x) = \frac{15120}{n^2}.$$

For the practical application of the function  $x$  in testing asymmetry we shall now require to construct a function of  $x$  which, as far as terms in  $n^{-2}$ , is distributed normally. Putting

$$x = \beta\xi + \delta(\xi^3 - 3\xi) + \eta(\xi^5 - 10\xi^3 + 15),$$

where  $\xi$  is normally distributed with unit variance, it is easy to obtain

$$\kappa_2(x) = \beta^2 + 6\delta^2,$$

$$\kappa_4(x) = 24\beta^4\delta + 216\beta^2\delta^2,$$

$$\kappa_6(x) = 720\beta^6\eta + 3240\beta^4\delta^2,$$

which are satisfied by

$$\beta = 1 - \frac{3}{n} - \frac{1}{4n^2}, \quad \delta = \frac{3}{2n} \left(1 - \frac{81}{2n}\right), \quad \eta = \frac{87}{8n^2},$$

or, inverting the relation between  $\xi$  and  $x$ , we have

$$\xi = x \left(1 + \frac{3}{n} + \frac{91}{4n^2}\right) - \frac{3}{2n} \left(1 - \frac{111}{2n}\right) (x^3 - 3x) - \frac{33}{8n^2} (x^5 - 10x^3 + 15x).$$

This translation formula makes it possible to assess the numerical effects upon tests of significance of the actual distribution; Tables 2 and 3 show the values of various possible formulae for the test deviate in the region, important for tests of significance,  $x = 1.8$  to  $2.2$ , and indicate that these effects are very serious.

TABLE 2.  
Comparison of deviates in five formulae for testing asymmetry.  
 $n = 100.$

(a)	(b)	(c)	(d)	(e)
$\sqrt{\frac{n}{6}} m_3 m_2^{-\frac{3}{2}}$	$\sqrt{\frac{n}{6}} k_3 k_2^{-\frac{3}{2}}$	$x$	$\xi_1$	$\xi_2$
1.7999	1.8274	1.8	1.8475	1.8603
1.9999	2.0305	2.0	2.0300	2.0586
2.1999	2.2335	2.2	2.2053	2.2530

TABLE 3.  
Comparison of deviates in five formulae for testing asymmetry.  
 $n = 50.$

(a)	(b)	(c)	(d)	(e)
$\sqrt{\frac{n}{6}} m_3 m_2^{-\frac{3}{2}}$	$\sqrt{\frac{n}{6}} k_3 k_2^{-\frac{3}{2}}$	$x$	$\xi_1$	$\xi_2$
1.7996	1.8558	1.8	1.8950	1.9463
1.9996	2.0620	2.0	2.0600	2.1745
2.1995	2.2682	2.2	2.2106	2.4016

In this region an error of 0.1 in the deviate produces an error of about 24 per cent. in the probability deduced, and, although high accuracy in the latter is not a necessity, little reliance can be placed upon tests when the deviate may be biased by as much as 0.2. Of the formulae tested, the formula (a) in terms of crude moments is almost equivalent to the use of  $x$ , and these are evidently the most in error. Of the simple formulae (b) is least in error, and for samples of 100 this error is only about .03. The value  $\xi_1$  shows the effect of using terms of the first degree only in the translation formula, while  $\xi_2$  shows the effect of using also terms in  $n^{-2}$ . There is evidently little to be gained by using  $\xi_1$  instead of the simple formula  $\sqrt{(n/6)} k_3 k_4^{-\frac{3}{2}}$ , which latter gives

apparently the better values for deviations exceeding 2.0. For samples as small as 50 the fully corrected value  $\xi_2$  is evidently required, and in view of the uncertainty of the effect of the omitted terms in  $n^{-2}$ , etc., no reliable test of normality for materially smaller samples can be said to be available. As in so many other cases, the adequate treatment even of moderately small samples is not well approached by series in  $n^{-1}$ .

## 12. The significance of the fourth moment.

The sampling variance of  $k_4$  from a normal sample is

$$\frac{24n(n+1)}{(n-1)(n-2)(n-3)} \kappa_2^4;$$

in testing the significance of such a value, we should therefore naturally calculate

$$x = \sqrt{\left( \frac{(n-1)(n-2)(n-3)}{24n(n+1)} \right) k_4 k_2^{-2}}$$

as a variate which, with increasing sample number, tends to be normally distributed with unit variance. With finite samples the distribution is asymmetrical, for  $\kappa(4^3)$  is not zero. The true mean value of  $x$  is zero, for with a normal distribution  $\kappa(4^2 p)$  is zero for all values of  $p$ , whence it follows that the mean of  $k_4$  is zero independently for all values of  $k_2$ .

The mean value of  $x^2$  is easily expanded in the form

$$\frac{(n-1)(n-2)(n-3)}{24n(n+1) \kappa_2^4} \left\{ \kappa(4^2) - \frac{4}{\kappa_2} \kappa(4^2 2) + \frac{10}{\kappa_2^2} \kappa(4^2) \kappa(2^2) + \dots \right\}$$

or 
$$1 - \frac{32}{n-1} + \frac{20}{n-1}$$

as far as  $n^{-1}$ .

The mean value of  $x^3$ , as far as  $n^{-\frac{3}{2}}$ , is

$$\left\{ \frac{(n-1)(n-2)(n-3)}{24n(n+1) \kappa_2^4} \right\}^{\frac{3}{2}} \left\{ \kappa(4^3) - \frac{6}{\kappa_2} \kappa(4^3 2) + \frac{21}{\kappa_2^2} \kappa(4^3) \kappa(2^2) + \dots \right\}$$

Now  $\kappa(4^3)$  has been evaluated by the direct combinatorial method, giving (formula 57)

$$\kappa(4^3) = \frac{1728n(n+1)(n^2-5n+2)}{(n-1)^2(n-2)2(n-3)^2} \kappa_2^6,$$

or, as near as needed,

$$\frac{1728}{n^8} (n+8);$$

while the leading term of  $\kappa(4^3 2)$  is  $1728n^{-3} \times 12$ , giving, as the mean value of  $x^3$ ,

$$\left\{ \frac{(n-1)(n-2)(n-3)}{n^3(n+1)} \right\}^{\frac{3}{2}} 6\sqrt{6} \{n+8-72+42\},$$

or 
$$\frac{6\sqrt{6}}{\sqrt{n}} \left(1 - \frac{65}{2n}\right).$$

Next, the mean value of  $x^4$  is, as far as  $n^{-1}$ ,

$$\left\{ \frac{(n-1)(n-2)(n-3)}{24n(n+1)\kappa_2^4} \right\}^2 \left\{ 3\kappa^2(4^2) + \kappa(4^4) - \frac{8}{\kappa_2} \{6\kappa(4^2)\kappa(4^2 2)\} + \frac{96}{\kappa_2^2} \{3\kappa^2(4^2)\kappa(2^2)\} \right\};$$

whence, subtracting three times the square of the mean of  $x^2$ , there remains

$$\kappa_4(x) = \left\{ \frac{(n-1)(n-2)(n-3)}{24n(n+1)\kappa_2^2} \right\}^2 \left\{ \kappa(4^4) - \frac{24}{\kappa_2} \kappa(4^2)\kappa(4^2 2) + \frac{96}{n-1} \kappa^2(4^2) \right\}.$$

The leading term in  $\kappa(4^4) \div 576\kappa_2^3$  comes to  $636n^{-3}$ , to which the other terms add  $-192$  and  $+96$  respectively, leaving

$$\kappa_4(x) = \frac{540}{n}.$$

For the mean value of  $x^5$  we shall need

$$\left( \frac{n}{24\kappa_2^4} \right)^{\frac{5}{2}} \left\{ 10\kappa(4^2)\kappa(4^3) + \kappa(4^5) - \frac{10}{\kappa_2} 10\kappa(4^2 2)\kappa(4^3) + 10\kappa(4^2)\kappa(4^2)\kappa(4^3 2) + \frac{550}{\kappa_2^2} \kappa(4^3)\kappa(4^2)\kappa(2^2) \right\},$$

whence, deducting  $10\kappa_2(x) \cdot \kappa_3(x)$ , there remains

$$\kappa_5(x) = \left( \frac{n}{24\kappa_2^4} \right)^{\frac{5}{2}} \left\{ \kappa(4^5) - \frac{60}{\kappa_2} \kappa(4^3)\kappa(4^2 2) - \frac{40}{\kappa_2} \kappa(4^3 2)\kappa(4^2) + \frac{240}{\kappa_2^2} \kappa(4^3)\kappa(4^2)\kappa(2^2) \right\},$$

or 
$$\frac{91}{n^{\frac{5}{2}}} \cdot 144\sqrt{6}.$$

Now if  $\xi$  is normally distributed with unit variance, and  $x$  can be expressed approximately in the form

$$x = \beta\xi + \gamma(\xi^2 - 1) + \delta(\xi^3 - 3\xi) + \epsilon(\xi^4 - 6\xi^2 + 3) + \dots,$$

we have

$$\kappa_1(x) = \mu_1(x) = 0,$$

$$\kappa_2(x) = \mu_2(x) = \beta^2 + 2\gamma^2 + 6\delta^2 + 24\epsilon^2 + \dots,$$

$$\kappa_3(x) = \mu_3(x) = 6\beta^2\gamma + 8\gamma^3 + 36\beta\gamma\delta + \dots,$$

$$\mu_4(x) = 3\beta^4 + 24\beta^3\delta + 48\beta^2\gamma^2,$$

whence

$$\kappa_4(x) = 24\beta^3\delta + 48\beta^2\gamma^2,$$

and

$$\mu_5(x) = 60\beta^4\gamma + 120\beta^4\epsilon + 1080\beta^3\gamma\delta + 680\beta^2\gamma^3,$$

whence

$$\kappa_5(x) = 120\beta^4\epsilon + 720\beta^3\gamma\delta + 560\beta^2\gamma^3;$$

and equating these to the actual values, neglecting  $n^{-2}$ , we have the translation formula

$$x = \left(1 - \frac{12}{n}\right) \xi + \sqrt{\frac{6}{n}} \left(1 - \frac{159}{2n}\right) (\xi^2 - 1) \\ + \frac{21}{2n} (\xi^3 - 3\xi) + \frac{91\sqrt{6}}{5n^{\frac{3}{2}}} (\xi^4 - 6\xi^2 + 3),$$

or, inversely,

$$\xi = -\frac{21\sqrt{6}}{n^{\frac{3}{2}}} + x \left(1 + \frac{36}{n}\right) - \sqrt{\frac{6}{n}} \left(1 - \frac{201}{2n}\right) (x^2 - 1) \\ + \frac{3}{2n} (x^3 - 3x) + \frac{43}{10n} \sqrt{\frac{6}{n}} (x^4 - 6x^2 + 3).$$

### Summary.

The equations which connect the moment functions of the sampling distribution of moment statistics with the moment functions of the population from which the samples are drawn correspond in univariate problems to all the partitions of all the natural numbers, and in multivariate problems to all the partitions of all multipartite numbers. Very few of this system of equations have hitherto been obtained owing to the algebraical complexity of their direct evaluation. The formulae are very much simplified (i) by using the semi-invariants instead of the moments of the population, and (ii) by using the system of moment statistics, the mean sampling value of each of which is the corresponding semi-invariant. The relations which necessarily exist between the different multivariate formulae demonstrate that all of these, as well as the univariate formulae, must be derivable from a system of rules associating

different two-way partitions of multipartite and unipartite numbers with corresponding functions of the sample number  $n$ .

Rules are given and illustrated which enable any term of any of these formulae to be obtained directly from an examination of the appropriate partition. Their general validity is demonstrated by a theorem which connects the moment generating function of any distribution with the corresponding function of any functionally related set of variates. Complete univariate formulae are given up to the tenth degree, and some new results are applied to the theory of samples from a normal population.

### References.

1. W. F. Sheppard, "On the application of the theory of error to cases of normal distribution and normal correlation", *Phil. Trans. (A)*, 192 (1897), 161-167.
2. K. Pearson, "On the probable errors of frequency constants", *Biometrika*, 2 (1902), 273-281.
3. K. Pearson, "On the probable errors of frequency constants", *Biometrika*, 9 (1913), 1-10.
4. L. Isserlis, "On certain probable errors and correlation coefficients of multiple frequency distribution with skew regression", *Biometrika*, 11 (1916), 185-190.
5. L. Isserlis, "Formulae for determining the mean value of products of deviations of mixed moment coefficients in two to eight variables in samples from a limited population", *Biometrika*, 12 (1919), 183-184.
6. H. E. Soper, "On the probable error of the correlation coefficient to the second approximation", *Biometrika*, 9 (1913), 91-115.
7. "Student", "The probable error of a mean", *Biometrika*, 6 (1908), 1-25.
8. A. A. Tchouproff, "On the mathematical expectation of the moments of frequency distributions", *Biometrika*, 12 (1919), 140-169 and 185-210.
9. A. E. R. Church, "On the moments of the distribution of squared standard deviations for samples of  $n$  drawn from an indefinitely large population", *Biometrika*, 17 (1925), 79-83.
10. R. A. Fisher, "On the mathematical foundations of theoretical statistics", *Phil. Trans. (A)*, 222 (1921), 309-368.
11. J. Wishart, "A problem in combinatorial analysis giving the distribution of certain moment statistics", *Proc. London Math. Soc. (2)*, 29 (1929), 309-321.





# A PROBLEM IN COMBINATORIAL ANALYSIS GIVING THE DISTRIBUTION OF CERTAIN MOMENT STATISTICS

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## 1. *Statement of the problem.*

It is natural, in studying problems of statistical theory, to regard any experience or group of events as only a sample out of a larger population of events. This population we may assume (a) to be infinite, (b) to follow a known mathematical law, such as the normal. It may be specified by citing the appropriate moment coefficients; thus, if normal and univariate, the mean and the standard deviation are sufficient to specify a particular distribution. With a number of variates it is necessary to know, not only all the means and standard deviations, but also the coefficients of correlation between any two variates. Speaking generally, we require all the product moment coefficients of the second degree. Our only knowledge of such a population comes from the sample, which is of finite size and is subject to random sampling errors. From the sample we can calculate, in any agreed manner, estimates of the population moment coefficients, which will be more or less inexact. One naturally finds that more reliance can be placed on a large than on a small sample. But whatever its size it is unsafe to take a sample estimate as furnishing the required population value without determining the errors to which the estimates are subject. A complete knowledge regarding these can be obtained if the frequency distribution, in samples, of the various moment coefficients can be determined theoretically. Much modern research has lain in this direction, and the distributions of the mean, standard deviation, and correlation and regression coefficients are known. A very general result of this nature has been put forward in a recent paper\*, and is as follows: Let  $\sigma_p$ ,  $\sigma_q$  and  $\rho_{pq}$  be typical standard deviations and coefficient

\* *Biometrika*, 20A (1928), 38.

of correlation of an  $n$ -variate population. Let  $\Delta$  be the determinant

$$|\rho_{pq}| \quad (p, q = 1, 2, \dots, n),$$

and let  $\Delta_{pq}$  be the minor of  $\rho_{pq}$  in  $\Delta$ . Let

$$a_{pq} = s_p s_q r_{pq} = \frac{\sum_1^N (x_p x_q)}{N}$$

be the product moment statistic of the  $p$ -th and  $q$ -th variates in a sample of size  $N$  from this population,  $x$  being the observed deviation from the mean. Then if we write

$$A_{pq} = A_{qp} = N \cdot \Delta_{pq} / 2\sigma_p \sigma_q \Delta,$$

we find that the simultaneous distribution, in random samples of  $N$ , of the  $n$  variances (squared standard deviations) and the  $\frac{1}{2}n(n-1)$  product moment coefficients, when the population sampled is normal, follows the law given by the differential relation

$$dp = \frac{|A_{11}, A_{22}, \dots, A_{nn}|^{\frac{1}{2}(N-1)}}{(\sqrt{\pi})^{\frac{1}{2}n(n-1)} \Gamma(\frac{1}{2}N - \frac{1}{2}) \Gamma(\frac{1}{2}N - 1) \dots \Gamma(\frac{1}{2}N - \frac{1}{2}n)} \\ \times \exp \left\{ - \sum_{p,q=1}^n A_{pq} a_{pq} \right\} |a_{11}, a_{22}, \dots, a_{nn}|^{\frac{1}{2}(N-n-2)} da_{11} da_{22} \dots da_{nn}, \quad (1)$$

in which the determinants are of the  $n$ -th order in both cases, with  $A_{pq}$  and  $a_{pq}$  respectively as typical elements.

A very great deal of information is contained within this general result. Thus the distribution, for one variate, of the mean and standard deviation, and, for two variates, of the correlation and regression coefficients, can be deduced directly from particular cases of (1)\*. It seems likely that new distributions for three or more variates will in time follow. Meantime, as a preparatory step in the direction of elucidating the required information, all the independent moment coefficients, up to the fourth order and eight variates, were worked out. The method first used was a generalization of a formula of Romanovsky†, and will be illustrated for the special case of three variates. Let

$$\mu' \begin{pmatrix} j & q & p \\ & k & m \\ & & l \end{pmatrix}$$

represent the general product moment coefficient, about an arbitrary origin, of the  $j$ -th,  $k$ -th and  $l$ -th powers of the three variances, and the

\* For historical references see the paper already cited.

† *Comptes rendus*, 180 (1925), 1898.

$m$ -th,  $p$ -th and  $q$ -th powers of the three product moment statistics. Then

$$\mu' \begin{pmatrix} j & q & p \\ k & m & l \end{pmatrix} = \left[ \frac{\partial^{j+k+l+m+p+q} \phi(\alpha\beta\gamma\lambda\mu\nu)}{\partial \alpha^j \partial \beta^k \partial \gamma^l \partial \lambda^m \partial \mu^p \partial \nu^q} \right]_{\alpha=\beta=\gamma=\lambda, \mu=\nu=0} \quad (2)$$

and

$$\phi(\alpha\beta\gamma\lambda\mu\nu) = \begin{vmatrix} A_{11} & A_{12} & A_{13} & A_{11}-\alpha & A_{12}-\frac{1}{2}\nu & A_{13}-\frac{1}{2}\mu \\ A_{21} & A_{22} & A_{23} & A_{21}-\frac{1}{2}\nu & A_{22}-\beta & A_{23}-\frac{1}{2}\lambda \\ A_{31} & A_{32} & A_{33} & A_{31}-\frac{1}{2}\mu & A_{32}-\frac{1}{2}\lambda & A_{33}-\gamma \end{vmatrix}^{-\frac{1}{2}(N-1)} \quad (3)$$

The general moment coefficient is thus expressed in terms of  $N$ , the size of the sample, and the following moment coefficients of the sampled population

$$\begin{aligned} \mu_{200} &= \sigma_1^2, & \mu_{020} &= \sigma_2^2, & \mu_{002} &= \sigma_3^2, \\ \mu_{110} &= \sigma_1 \sigma_2 \rho_{12}, & \mu_{101} &= \sigma_1 \sigma_3 \rho_{13}, & \mu_{011} &= \sigma_2 \sigma_3 \rho_{23}. \end{aligned}$$

The corresponding general moment coefficients, when taken about their own mean value in samples as origin, will be designated by

$$\mu \begin{pmatrix} j & q & p \\ k & m & l \end{pmatrix}.$$

It was soon evident, however, that, in all but the simplest cases, the labour attendant on the direct calculation by means of (2) of the 111 independent moments up to the fourth order would be very great. Moreover, the logical order of procedure is to calculate the general 4, 6 and 8 variate results

$$\mu \begin{pmatrix} 1 & \cdot & \cdot \\ \cdot & 1 & \cdot \\ \cdot & \cdot & 1 \end{pmatrix}, \quad \mu \begin{pmatrix} 1 & \cdot & \cdot & \cdot \\ \cdot & 1 & \cdot & \cdot \\ \cdot & \cdot & 1 & \cdot \\ \cdot & \cdot & \cdot & 1 \end{pmatrix}, \quad \mu \begin{pmatrix} 1 & \cdot & \cdot & \cdot & \cdot \\ \cdot & 1 & \cdot & \cdot & \cdot \\ \cdot & \cdot & 1 & \cdot & \cdot \\ \cdot & \cdot & \cdot & 1 & \cdot \\ \cdot & \cdot & \cdot & \cdot & 1 \end{pmatrix},$$

from which, by associating the numbers 1 to 8 in all the possible ways, all the 2nd order, 3rd order, and 4th order formulae respectively can be reached. Such calculations would, however, be exceedingly difficult by the procedure outlined. At this stage the author was prompted, on the suggestion of Dr. R. A. Fisher, to consider his problem as a special case of the application, to general sampling theory, of the methods of combinatorial analysis. In what follows the correspondence between the two theories will be sketched, and the importance of the study of the partitions of unipartite and multipartite numbers in the theory of statistics will be indicated.

## 2. Cumulative moment functions.

The expressions which can be determined by an application of combinatorial analysis are not, however, the moments themselves, but the corresponding cumulative moment functions. These functions are identical with the semi-invariants of Thiele, and the applications to be discussed, together with the simplicity and elegance of the resulting formulae, combine to give an added importance to functions originally introduced for quite a different reason.

If the probability that a single sample value falls within the infinitesimal range  $dx$  is  $\phi(x)dx$ , so that

$$\int_{-\infty}^{\infty} \phi(x) dx = 1,$$

then the "characteristic function", or moment generating function,  $M$ , is defined by the relation

$$M = \int_{-\infty}^{\infty} e^{ix} \phi(x) dx.$$

This function is always well defined for all pure imaginary values of  $t^*$ . Even should certain of the moments of the distribution, i.e.

$$\int_{-\infty}^{\infty} x^r \phi(x) dx,$$

become infinite,  $M$  is still well defined and  $|M| \leq 1$  (since  $|e^{ix}| = 1$ ).  $M$  is therefore absolutely convergent: it is, in fact, an integral of Stieltjes, which, when the moments as defined below have a meaning, can be expanded at the origin in the form

$$\begin{aligned} M &= 1 + \mu'_1 t + \mu'_2 \frac{t^2}{2!} + \mu'_3 \frac{t^3}{3!} + \dots \\ &= e^{\mu'_1 t} \left[ 1 + \mu_2 \frac{t^2}{2!} + \mu_3 \frac{t^3}{3!} + \dots \right], \end{aligned}$$

where we have written, in the usual notation,  $\mu'_r$  for

$$\int_{-\infty}^{\infty} x^r \phi(x) dx,$$

and  $\mu_r$  for the corresponding moment about the mean of the distribution. If the logarithm of  $M$  can now be expanded in powers of  $t$ , we have

$$K = \log M = \kappa_1 t + \kappa_2 \frac{t^2}{2!} + \kappa_3 \frac{t^3}{3!} + \dots$$

\* See P. Lévy, *Calcul des probabilités* (Gauthier-Villars, 1925), 161, 162, 172, et seq.

as the generating function of the cumulative moment functions  $\kappa$ . The  $\kappa$ 's are determinate functions of the  $\mu$ 's, and can be obtained in terms of these by equating coefficients of like powers of  $t$ . It is sufficient for our present purpose to note that

$$\kappa_1 = \mu'_1, \quad \kappa_2 = \mu_2, \quad \kappa_3 = \mu_3, \quad \kappa_4 = \mu_4 - 3\mu_2^2, \quad (4)$$

formulae which enable us to convert  $\mu$ 's into  $\kappa$ 's and *vice versa*. Since the relations are invariant and independent of the nature of the distribution, the case where the logarithm of  $M$  cannot be so expanded need not be considered.

Similar relations exist when two or more variates fall to be considered. Thus, for a bi-variate system, corresponding to the *moment generating function*

$$\begin{aligned} M &= \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} e^{x t_1 + y t_2} \phi(xy) dx dy \\ &= 1 + \mu'_{10} t_1 + \mu'_{01} t_2 + \mu'_{20} \frac{t_1^2}{2!} + 2\mu'_{11} \frac{t_1 t_2}{2!} + \mu'_{02} \frac{t_2^2}{2!} + \mu'_{30} \frac{t_1^3}{3!} + 3\mu'_{21} \frac{t_1^2 t_2}{3!} + \dots \\ &= e^{\mu'_{10} t_1 + \mu'_{01} t_2} \left[ 1 + \mu_{20} \frac{t_1^2}{2!} + 2\mu_{11} \frac{t_1 t_2}{2!} + \mu_{02} \frac{t_2^2}{2!} + \mu_{30} \frac{t_1^3}{3!} + 3\mu_{21} \frac{t_1^2 t_2}{3!} + \dots \right], \end{aligned}$$

we have a *kappa generating function*

$$K = \log M = \kappa_{10} t_1 + \kappa_{01} t_2 + \kappa_{20} \frac{t_1^2}{2!} + 2\kappa_{11} \frac{t_1 t_2}{2!} + \kappa_{02} \frac{t_2^2}{2!} + \dots$$

The  $\mu$ 's and the  $\kappa$ 's agree up to the third order, by virtue of the origin being at the mean, while, for the fourth order,

$$\left. \begin{aligned} \kappa_{40} &= \mu_{40} - 3\mu_{20}^2, & \kappa_{04} &= \mu_{04} - 3\mu_{02}^2 \\ \kappa_{31} &= \mu_{31} - 3\mu_{20}\mu_{11}, & \kappa_{13} &= \mu_{13} - 3\mu_{02}\mu_{11} \\ \kappa_{22} &= \mu_{22} - \mu_{20}\mu_{02} - 2\mu_{11}^2 \end{aligned} \right\}. \quad (5)$$

These formulae are, in fact, particular cases of the following one, which is all that need be remembered :

$$\kappa_{1111} = \mu_{1111} - \mu_{1100}\mu_{0011} - \mu_{1010}\mu_{0101} - \mu_{1001}\mu_{0110}. \quad (6)$$

Any number of noughts can be inserted in the suffixes of this formula, while by the association of two or more of the one's, all the special cases that are likely to arise, such as equations (5), can be deduced. Similar formulae give the  $\mu$ 's in terms of the  $\kappa$ 's, but in this case all the terms are positive. Thus the reverse of (6) is

$$\mu_{1111} = \kappa_{1111} + \kappa_{1100}\kappa_{0011} + \kappa_{1010}\kappa_{0101} + \kappa_{1001}\kappa_{0110}. \quad (7)$$

The generating functions, introduced in this section, play a very useful rôle in the study of frequency distributions, since they present all the moments in an orderly scheme to the eye, and capable of transformation as required. Further, for the particular case of the normal distribution the kappa generating function degenerates into its first two terms, since all  $\kappa$ 's beyond  $\kappa_2$  vanish.

### 3. Determination of sampling moments—two variates.

Turning now to the determination of the cumulative moment functions corresponding to the moments given by (2), we may conveniently begin by writing down the well known results for the cumulative moment functions of the variance

$$\begin{aligned}\kappa(1) &= (N-1)\kappa_2/N = (N-1)\sigma^2/N, & \kappa(2) &= 2(N-1)\kappa_2^2/N^2, \\ \kappa(3) &= 8(N-1)\kappa_2^3/N^3, & \kappa(4) &= 48(N-1)\kappa_2^4/N^4,\end{aligned}$$

the general result being

$$\kappa(s) = 2^{s-1}(s-1)!(N-1)\kappa_2^s/N^s,$$

which is directly derivable from the exact distribution of the variance from a normal sample

$$dp = A^{\frac{1}{2}(N-1)} e^{-Aa} a^{\frac{1}{2}(N-3)} da / \Gamma(\frac{1}{2}N - \frac{1}{2}),$$

in the notation of the early part of this paper.

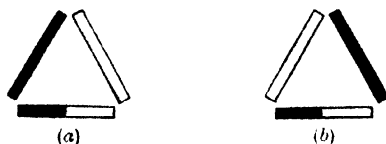
A study of the rules\* which govern the numerical coefficients in the case of moment statistics of any order derived in general from **non-normal** populations shows that the above coefficients should be regarded as the number of ways in which the multipartite numbers (2), (2<sup>2</sup>), (2<sup>3</sup>), ..., (2<sup>s</sup>) can be partitioned into  $s$  parts of two units each; the restriction that we shall consider only those partitions in which the elements of the multipartite number are linked together to form a complete ring guarantees the correctness of the numerical coefficients. It is only in the case of the cumulative moment functions that these simple coefficients, both the numerical ones and those in terms of  $N$ , occur; while the actual form of the general coefficient indicates that it is these functions, and these alone, which are determined by the partitional problem about to be described. In this representation the problem of finding, for two variates, the coefficient of

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\* I owe the use of certain empirical conclusions which are made at this stage to information derived from Dr. R. A. Fisher, who felt justified in drawing them in consequence of some investigations which he has made but which are as yet unpublished. A general demonstration of these rules has since been obtained by Dr. Fisher, and will appear shortly in these *Proceedings*.

$(N-1) \kappa_{20}^p \kappa_{11}^q \kappa_{02}^r / N^s$  in  $\kappa \left( \begin{smallmatrix} l & m \\ n \end{smallmatrix} \right)$  will be equivalent to the problem of finding the number of ways of arranging in a ring  $l$  rods, of which both ends are black,  $n$ , of which both are white, and  $m$  with one black and one white end, in such a way that there are  $p$  junctions black to black,  $r$  junctions white to white, and  $q$  junctions black to white. Evidently  $p+q+r=l+m+n=s$ , and also  $p-r=l-n$ , so that the complete expression for  $\kappa \left( \begin{smallmatrix} l & m \\ n \end{smallmatrix} \right)$  will consist of a single series of terms with coefficients determined by the combinatorial problem, their sum being  $2^{s-1}(s-1)!$ .

To give two simple examples,  $\kappa \left( \begin{smallmatrix} 1 & 1 \\ 1 \end{smallmatrix} \right)$  will be found by considering the possible arrangements in a ring of three rods, black-black, white-white, and black-white respectively; only two essentially different arrangements are possible, as shown below:



Each of these can be set up in four ways; arrangement (a) has only one unlike junction and supplies the term

$$4 \frac{N-1}{N^3} \kappa_{20} \kappa_{11} \kappa_{02};$$

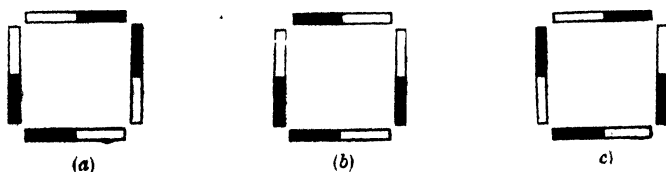
arrangement (b) has three unlike junctions and supplies the term

$$4 \frac{N-1}{N^3} \kappa_{11}^3.$$

The intuitive solution of the combinatorial problem thus leads to the complete expression

$$\kappa \left( \begin{smallmatrix} 1 & 1 \\ 1 \end{smallmatrix} \right) = 4 \frac{N-1}{N^3} \kappa_{11} (\kappa_{20} \kappa_{02} + \kappa_{11}^2) = 4 \frac{N-1}{N^3} \sigma_1^3 \sigma_2^3 \rho (1 + \rho^2).$$

As an example with four rods, consider  $\kappa \left( \begin{smallmatrix} \cdot & 4 \\ \cdot \end{smallmatrix} \right)$ ; here the rods are all black-white, and three essentially different arrangements are possible:



Of these (a) may be set up in six ways, (b) in thirty-six ways, and (c) in six ways, giving

$$\begin{aligned}\kappa\left(\begin{smallmatrix} \cdot & 4 \\ & \cdot \end{smallmatrix}\right) &= 6 \frac{N-1}{N^4} (\kappa_{20}^2 \kappa_{02}^2 + 6\kappa_{20} \kappa_{02} \kappa_{11}^2 + \kappa_{11}^4) \\ &= 6 \frac{N-1}{N^4} \sigma_1^4 \sigma_2^4 (1 + 6\rho^2 + \rho^4).\end{aligned}$$

These two results are identical with those found from a direct application of (2), correcting in the usual way for the mean.

#### 4. Operational solution.

The foregoing rules enable us to determine the simplest of the required formulae very quickly. It then becomes evident that higher order results can be deduced from those already calculated simply by considering the number of ways in which an additional rod can be inserted into the ring. Suppose that we desire to increase  $l$  by unity, leaving all else unchanged in a moment of the  $s$ -th order. A rod can be introduced in two ways at each of the  $s$  junctions, showing that the sum of the numerical coefficients is multiplied by  $2s$ . The effect it has upon the character of the result depends on the particular junction. Inserted between two blacks, it has the effect of wiping out the black-black junction and adding two new ones. If placed between two whites, the white-white junction is eliminated and two black-white junctions are substituted. Finally, if the old junction is black-white this is replaced by a black-black and a black-white. Remembering that the new rod may be turned round, we have the whole change described symbolically by means of the following operator :

(a) To increase  $l$  by unity :

$$\Omega\left(\begin{smallmatrix} 1 & \cdot \\ & \cdot \end{smallmatrix}\right) = 2\kappa_{20}^2 \frac{d}{d\kappa_{20}} + 2\kappa_{20} \kappa_{11} \frac{d}{d\kappa_{11}} + 2\kappa_{11}^2 \frac{d}{d\kappa_{02}}.$$

(b) Similarly, to increase  $n$  by unity :

$$\Omega\left(\begin{smallmatrix} \cdot & \cdot \\ & 1 \end{smallmatrix}\right) = 2\kappa_{11}^2 \frac{d}{d\kappa_{20}} + 2\kappa_{02} \kappa_{11} \frac{d}{d\kappa_{11}} + 2\kappa_{02}^2 \frac{d}{d\kappa_{02}}.$$

Finally, if the rod to be inserted is black-white, a double-black junction is replaced by a double-black and a black-white, a double-white junction becomes a double-white plus a black-white; but for the black-white junction there are two possibilities, according to the way in which the rod is inserted. In one case black-white is eliminated and

black-black, white-white substituted: and in the other two white-blacks are formed from one. This corresponds to the operator

(c) To increase  $m$  by unity:

$$\begin{aligned}\Omega\left(\begin{smallmatrix} 1 \\ \cdot \\ 1 \end{smallmatrix}\right) &= (\kappa_{20}\kappa_{02} + \kappa_{11}^2) \frac{d}{d\kappa_{11}} + 2\kappa_{20}\kappa_{11} \frac{d}{d\kappa_{20}} + 2\kappa_{02}\kappa_{11} \frac{d}{d\kappa_{02}} \\ &= (\kappa_{20}\kappa_{02} + \kappa_{11}^2) \frac{d}{d\kappa_{11}} + 2\kappa_{11} \sqrt{(\kappa_{20}\kappa_{02})} \frac{d}{d\sqrt{(\kappa_{20}\kappa_{02})}}.\end{aligned}$$

The operators (a), (b) and (c) enable us without difficulty to write down quite complex results. The arithmetical procedure is simple, and is best exhibited as in the following illustrative scheme, where  $\kappa\left(\begin{smallmatrix} 4 & 5 \\ & 3 \end{smallmatrix}\right)$  is evaluated from the simple result

$$\kappa\left(\begin{smallmatrix} 4 & \cdot \\ & 1 \end{smallmatrix}\right) = 2^4 \cdot 4! \frac{N-1}{N^5} \kappa_{20}^3 \kappa_{11}^2 = 2^4 \cdot 4! \frac{N-1}{N^5} \sigma_1^8 \sigma_2^2 \rho^2.$$

$\kappa$	Coefft.	Index of $\kappa_{11}$ .											
		0	1	2	3	4	5	6	7	8	9	10	11
$\left(\begin{smallmatrix} 4 & 0 \\ & 1 \end{smallmatrix}\right)$	$2^7 \cdot 3$	(i)		1									
$\left(\begin{smallmatrix} 4 & 0 \\ & 2 \end{smallmatrix}\right)$	$2^8 \cdot 3$			2		3							
$\left(\begin{smallmatrix} 4 & 0 \\ & 3 \end{smallmatrix}\right)$	$2^{10} \cdot 3^2$			1		3		1					
$\left(\begin{smallmatrix} 4 & 1 \\ & 3 \end{smallmatrix}\right)$	$2^{11} \cdot 3^2$	(ii)	1		12		18		4				
$\left(\begin{smallmatrix} 4 & 2 \\ & 3 \end{smallmatrix}\right)$	$2^{11} \cdot 3^2$		1		51		246		226		36		
$\left(\begin{smallmatrix} 4 & 3 \\ & 3 \end{smallmatrix}\right)$	$2^{14} \cdot 3^3 \cdot 5$			1		15		40		25		3	
$\left(\begin{smallmatrix} 4 & 4 \\ & 3 \end{smallmatrix}\right)$	$2^{14} \cdot 3^3 \cdot 5$		1		64		455		775		352		33
$\left(\begin{smallmatrix} 4 & 5 \\ & 3 \end{smallmatrix}\right)$	$2^{15} \cdot 3^3 \cdot 5$			75		1550		6420		7608		2629	198

$$\begin{aligned}\kappa\left(\begin{smallmatrix} 4 & 5 \\ & 3 \end{smallmatrix}\right) &= 2^{15} \cdot 3^3 \cdot 5 \frac{N-1}{N^{12}} \kappa_{20} \kappa_{11} (75 \kappa_{20}^5 \kappa_{02}^5 + 1550 \kappa_{20}^4 \kappa_{02}^4 \kappa_{11}^2 + 6420 \kappa_{20}^3 \kappa_{02}^3 \kappa_{11}^4 \\ &\quad + 7608 \kappa_{20}^2 \kappa_{02}^2 \kappa_{11}^6 + 2629 \kappa_{20} \kappa_{02} \kappa_{11}^8 + 198 \kappa_{11}^{10}) \\ &= 2^{15} \cdot 3^3 \cdot 5 \frac{N-1}{N^{12}} \sigma_1^{13} \sigma_2^{11} \rho (75 + 1550 \rho^2 + 6420 \rho^4 + 7608 \rho^6 \\ &\quad + 2629 \rho^8 + 198 \rho^{10}).\end{aligned}$$

(i) Here we apply formula (b). The first stage produces two terms, with coefficients  $2 \times 2$  and  $2 \times 3$ . These are doubled in the next stage and added to terms with coefficients  $2 \times 2$ ,  $3(2 \times 4)$ , and  $3(2 \times 2)$ . The nature of each term is indicated by the column in which it is situated.

(ii) The application of the operator (c) is equivalent to multiplying diagonally to the left by the index of  $\kappa_{11}$ , and then diagonally to the right by a complementary series, such that the sum of the multipliers of any one term is equal to twice the sum of  $l$ ,  $m$ , and  $n$  in that line. A common factor is divided out wherever it occurs.

It is evident that the entire scheme of product moments may be built up in the way illustrated, with the minimum of labour, where the direct application of formula (2) would be tedious. In fact, the labour attendant on the methods hitherto employed has, of necessity, confined attention solely to the direct moments and to the simplest of the product moments. In the case of the more general results for non-normal populations, too, it has been difficult in the past to check the results reached and detect errors\*. On the other hand, the use of the equivalent partitional problem is an immense addition to our practical knowledge of sampling moments, since it makes available any required moment of the distribution (1).

### 5. Extension to three or more variates.

When the sampled population has three variates, the ring arrangement will be built up of rods whose ends may be of three different colours, *e.g.* black, white, red. The *ensemble* of colours in any calculation will be determined by the particular sample moment which it is desired to evaluate, and, as before, any arrangement will specify, by means of the paired colours at the junctions, a particular combination of powers of the six population moment coefficients, thus determining one term of the required  $\kappa$ . The sum of the coefficients of all the terms that can arise, from consideration of all the possible arrangements, will again be  $2^{s-1}(s-1)!$ , where there are  $s$  rods. To give one example

$$\begin{aligned} \kappa \begin{pmatrix} 1 & 1 \\ & 1 \end{pmatrix} &= 2 \frac{N-1}{N^3} (\kappa_{200} \kappa_{002} \kappa_{110} + \kappa_{200} \kappa_{101} \kappa_{011} + 2\kappa_{110} \kappa_{101}^2) \\ &= 2 \frac{N-1}{N^3} \sigma_1^2 \sigma_2 \sigma_3^2 (\rho_{12} + \rho_{13} \rho_{23} + 2\rho_{12} \rho_{13}^2), \end{aligned}$$

the three possible arrangements of the rods being given below :



(a)



(b)



(c)

(a) may be set up in two ways, (b) in two ways, and (c) in four ways.

Consideration of the possible number of ways of introducing a new rod leads to the following operators, which can be used to deduce higher order results.

(a) To increase double-black by unity.

$$\Omega\left(\begin{smallmatrix} 1 & & \\ & \cdot & \\ & & \cdot \end{smallmatrix}\right) = 2\kappa_{200}^2 \frac{d}{d\kappa_{200}} + 2\kappa_{200} \kappa_{110} \frac{d}{d\kappa_{110}} + 2\kappa_{200} \kappa_{101} \frac{d}{d\kappa_{101}} \\ + 2\kappa_{110}^2 \frac{d}{d\kappa_{020}} + 2\kappa_{101}^2 \frac{d}{d\kappa_{012}} + 2\kappa_{110} \kappa_{101} \frac{d}{d\kappa_{011}}.$$

(b) and (c) To increase double white or double red by unity.

Similar to (a), and got from it by permuting the three suffixes.

(d) To increase black-white by unity.

$$\Omega\left(\begin{smallmatrix} \cdot & 1 & \\ & \cdot & \\ & & \cdot \end{smallmatrix}\right) = (\kappa_{200} \kappa_{020} + \kappa_{110}^2) \frac{d}{d\kappa_{110}} + (\kappa_{200} \kappa_{011} + \kappa_{110} \kappa_{101}) \frac{d}{d\kappa_{101}} \\ + (\kappa_{020} \kappa_{101} + \kappa_{110} \kappa_{011}) \frac{d}{d\kappa_{011}} + 2\kappa_{200} \kappa_{110} \frac{d}{d\kappa_{200}} \\ + 2\kappa_{020} \kappa_{110} \frac{d}{d\kappa_{020}} + 2\kappa_{101} \kappa_{011} \frac{d}{d\kappa_{002}}.$$

(e) and (f) To increase white-red or red-black by unity.

Obtained from (d) by permuting the three suffixes.

Generally, we may deal with the multi-variate case by supposing there to be as many different colours as there are variates. We are then enabled to present the general second, third, and fourth order cumulative moment functions by going up to a maximum of eight variates. It will make the exact correspondence between the two problems clear if we

write down these general results :

$$\kappa \begin{pmatrix} \cdot & 1 & \cdot & \cdot \\ & \cdot & \cdot & \cdot \\ & & 1 & \cdot \\ & & & 1 \end{pmatrix} = \frac{N-1}{N^2} (\kappa_{1010} \kappa_{0101} + \kappa_{1001} \kappa_{0110}) = \frac{N-1}{N^2} \sigma_1 \sigma_2 \sigma_3 \sigma_4 (\rho_{13} \rho_{24} + \rho_{14} \rho_{23}), \quad (8)$$

$$\kappa \begin{pmatrix} \cdot & 1 & \cdot & \cdot & \cdot & \cdot \\ & \cdot & \cdot & \cdot & \cdot & \cdot \\ & & 1 & \cdot & \cdot & \cdot \\ & & & \cdot & \cdot & \cdot \\ & & & & 1 & \cdot \\ & & & & & 1 \end{pmatrix} = \frac{N-1}{N^3} \sigma_1 \sigma_2 \sigma_3 \sigma_4 \sigma_5 \sigma_6 [\rho_{13}(\rho_{25} \rho_{46} + \rho_{26} \rho_{45}) + \rho_{14}(\rho_{25} \rho_{36} + \rho_{26} \rho_{35}) \\ + \rho_{15}(\rho_{23} \rho_{46} + \rho_{24} \rho_{36}) + \rho_{16}(\rho_{23} \rho_{45} + \rho_{24} \rho_{35})], \quad (9)$$

$$\kappa \begin{pmatrix} \cdot & 1 & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ & & 1 & \cdot & \cdot & \cdot & \cdot & \cdot \\ & & & \cdot & \cdot & \cdot & \cdot & \cdot \\ & & & & 1 & \cdot & \cdot & \cdot \\ & & & & & \cdot & \cdot & \cdot \\ & & & & & & 1 & \cdot \\ & & & & & & & 1 \end{pmatrix} = \frac{N-1}{N^4} \sigma_1 \sigma_2 \sigma_3 \sigma_4 \sigma_5 \sigma_6 \sigma_7 \sigma_8 \\ \times [(\rho_{13} \rho_{25} + \rho_{15} \rho_{23})(\rho_{47} \rho_{68} + \rho_{48} \rho_{67}) + (\rho_{13} \rho_{26} + \rho_{16} \rho_{23})(\rho_{47} \rho_{58} + \rho_{48} \rho_{57}) \\ + (\rho_{13} \rho_{27} + \rho_{17} \rho_{23})(\rho_{45} \rho_{68} + \rho_{46} \rho_{58}) + (\rho_{13} \rho_{28} + \rho_{18} \rho_{23})(\rho_{45} \rho_{67} + \rho_{46} \rho_{57}) \\ + (\rho_{14} \rho_{25} + \rho_{15} \rho_{24})(\rho_{37} \rho_{68} + \rho_{38} \rho_{67}) + (\rho_{14} \rho_{26} + \rho_{16} \rho_{24})(\rho_{37} \rho_{58} + \rho_{38} \rho_{57}) \\ + (\rho_{14} \rho_{27} + \rho_{17} \rho_{24})(\rho_{35} \rho_{68} + \rho_{36} \rho_{58}) + (\rho_{14} \rho_{28} + \rho_{18} \rho_{24})(\rho_{35} \rho_{67} + \rho_{36} \rho_{57}) \\ + (\rho_{15} \rho_{27} + \rho_{17} \rho_{25})(\rho_{36} \rho_{48} + \rho_{38} \rho_{46}) + (\rho_{15} \rho_{28} + \rho_{18} \rho_{25})(\rho_{36} \rho_{47} + \rho_{37} \rho_{46}) \\ + (\rho_{16} \rho_{27} + \rho_{17} \rho_{26})(\rho_{35} \rho_{48} + \rho_{38} \rho_{45}) + (\rho_{16} \rho_{28} + \rho_{18} \rho_{26})(\rho_{35} \rho_{47} + \rho_{37} \rho_{45})]. \quad (10)$$

Results (8) and (9) are equal to their corresponding  $\mu$ 's, while (10) can be converted, using (7), by adding the term

$$\frac{(N-1)^2}{N^4} \sigma_1 \sigma_2 \sigma_3 \sigma_4 \sigma_5 \sigma_6 \sigma_7 \sigma_8 \\ \times [(\rho_{13} \rho_{24} + \rho_{14} \rho_{23})(\rho_{57} \rho_{68} + \rho_{58} \rho_{67}) + (\rho_{15} \rho_{26} + \rho_{16} \rho_{25})(\rho_{37} \rho_{48} + \rho_{38} \rho_{47}) \\ + (\rho_{17} \rho_{28} + \rho_{18} \rho_{27})(\rho_{35} \rho_{46} + \rho_{36} \rho_{45})].$$

These results, particularly the higher order ones, are so complex that no practical statistician would dream of computing them. They are given here, as certain analogous results were once given by Isserlis\*, in order that the simpler results may be deduced by associating the numbers 1 to 8 in all possible ways. Thus, to select a third order result at

random, if we let  $5 = 6 = 1$ , in (9), we get

$$\kappa \begin{pmatrix} 1 & 1 & \cdot & \cdot \\ & \cdot & \cdot & \cdot \\ & & \cdot & 1 \end{pmatrix} = 2 \frac{N-1}{N^3} \sigma_1^2 \sigma_2 \sigma_3 \sigma_4 (\rho_{13} \rho_{24} + \rho_{14} \rho_{23} + 2\rho_{12} \rho_{13} \rho_{14}).$$

Again, the product moment coefficient of the four variances, got from (10) by putting  $5 = 1$ ,  $6 = 2$ ,  $7 = 3$ , and  $8 = 4$ , and adjusting by (7), is

$$\mu \begin{pmatrix} 1 & \cdot & \cdot & \cdot \\ & 1 & \cdot & \cdot \\ & & 1 & \cdot \\ & & & 1 \end{pmatrix} = 4 \frac{N-1}{N^4} \sigma_1^2 \sigma_2^2 \sigma_3^2 \sigma_4^2 [4(\rho_{12} \rho_{13} \rho_{24} \rho_{34} + \rho_{12} \rho_{14} \rho_{23} \rho_{34} + \rho_{13} \rho_{14} \rho_{23} \rho_{24}) \\ + (N-1)(\rho_{12}^2 \rho_{34}^2 + \rho_{13}^2 \rho_{24}^2 + \rho_{14}^2 \rho_{23}^2)].$$

But consideration of the partitional method obviates this indirect method of attack. For instead of working out the general result for all colours different and then associating two or more of these, it is easier to associate the colours first and then consider the simpler ring arrangement that now awaits solution. The utility of the method obviously lies along these lines.

## 6. General considerations.

The foregoing has dealt with one specific problem only, namely, the distribution of the second order moment statistics from a normal population. The correspondence with equivalent problems in the domain of combinatorial analysis has been indicated. It has since been established that the general problem for non-normal populations, and for moments higher than the second in the case of normal populations, is also intimately connected with combinatorial theory, and the special arrangement of rods in a ring has been of service by analogy in suggesting the more complicated forms of the correspondence between the two theories



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## SAMPLING ERRORS IN THE THEORY OF TWO FACTORS

By JOHN WISHART.

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THE mathematical implications of the two-factor theory have recently aroused a great deal of interest. Thus Prof. Spearman's book, *The Abilities of Man*, has been reviewed in *Nature* (6 August, 1927) and in *Science* (2 March, 1928), and the writers of the reviews have confined themselves almost solely to the mathematical appendix. Again, Prof. Pearson and Miss Moul, in *Biometrika*, xix, pp. 246-91, have criticized the theory on the ground that data adduced in support of it by Spearman fail to show up so well when critically examined by statistical methods. The truth, of course, is that the development of the two-factor theory has caught the statistician in a state of unpreparedness, for his own theory is not yet sufficiently advanced to enable him to deal adequately with such a complicated sampling problem as the two-factor theory presents. To suggest a new line of attack which may possibly carry us a stage further is the object of the present paper.

It is a necessary consequence of the theory of two factors that all the tetrad differences  $F$  should be zero. This is where the theory of statistics is invoked. All we can hope to examine in psychological practice is a sample, and that not always a large one, out of the total population. Even if the tetrad difference were zero in reality it is hardly likely that this value would be exactly realized from a sample, which could not be completely representative of the whole. Owing to the vagaries of random sampling a value, differing from zero, will be obtained, or if a number of abilities are measured for each individual, an average tetrad difference will be found. We have then to determine the probability that such a value should arise by chance in sampling from a population in which the tetrad differences are in fact zero. For the case of the correlation coefficient  $r$  this has been done by studying the exact distribution in samples of this coefficient, and by providing tables showing what values of  $r$  should be reached for various levels of significance<sup>1</sup>. But the distribution of the tetrad difference has not yet been ascertained.

<sup>1</sup> R. A. Fisher, *Statistical Methods for Research Workers*, 2nd ed. Oliver and Boyd, 1928.

Its standard deviation, however (*i.e.* the average of the squares of all the values of  $F$  that would be reached if the experiment were repeated a great many times), has been discussed. This parameter of the distribution indicates to some extent whether experimental results are compatible with the two-factor theory or not. Hitherto the standard deviation has only been known approximately in the form of one or two terms of an expansion proceeding in inverse powers of  $N$ , the number in the sample. But an exact formula is much to be preferred. If  $N$  is not really large the first term or two in an expansion will not be correct enough. A more serious objection is that in particular cases the early terms of a series may vanish, and the first term of importance may be a term neglected.

In the process of calculating the correlation coefficient for every pair of abilities we require to determine the mean value of the products of the numerical measures of, say, the  $p$ th and  $q$ th abilities. Let us specify this quantity by  $a_{pq}$ , the 'co-variance' (a convenient term which is used in this laboratory). For  $n$  abilities there are altogether  $\frac{1}{2}n(n+1)$  values of  $a_{pq}$ , including the cases where  $p=q$ , which are the variances ( $\sigma^2$ ). We now define a new quantity  $P'$ , the tetrad product, by the equation

$$P' = a_{13}a_{24} - a_{14}a_{23} \dots\dots\dots(1).$$

$P'$  is equal to  $F$  multiplied by the estimates obtained from the sample of the four standard deviations, and therefore vanishes with  $F$ . But whereas the standard deviation of  $F$  in samples is only known approximately, that of  $P'$  can be determined exactly. A former paper by the author<sup>1</sup> dealt with the simultaneous distribution in samples of all the quantities like  $a_{pq}$  that can be formed from  $n$  variates, and provided a table of the moments of this distribution. That is, the mean values of the products of any powers of the co-variances  $a_{pq}$  with one another were stated, as far as the fourth order and eight variates, and it was indicated how higher order results could be determined algebraically. As the tetrad product  $P'$  is a simple quadratic function of the  $a$ 's, the calculation of the moment coefficients of  $P'$  can be performed directly from the table. The first is the mean value of  $P'$  in many samples, while the second is the variance ( $\sigma_{P'}^2$ ). For the details of the proof the appendix should be consulted. One modification of our equation (1) suggests itself before we proceed very far. Let  $x_1$  be the deviation of the measure of ability 1 from the mean value for all the individuals. Similarly for  $x_2, x_3, x_4$ . Then

$$a_{13} = \frac{1}{N} S(x_1 x_3), \text{ etc.,}$$

<sup>1</sup>. *Biometrika*, **xx** A, 1928, pp. 32-52.

where the  $S$  stands for summation over all individuals. Let us calculate, not  $P'$ , but a quantity  $P$ , defined as follows:

$$P = \frac{1}{(N-1)(N-2)} [S(x_1x_3)S(x_2x_4) - S(x_1x_4)S(x_2x_3)] \dots\dots(2).$$

$P$  and  $P'$  are simply related, only differing in the ratio of  $(N-1)(N-2)$  to  $N^2$ . But the advantage of working with (2) is that the mean value of  $P$  in many samples is equal to the corresponding value of  $P$  (call it  $\Pi$ ) in the infinite population from which our sample is drawn. The other definition would have produced a biased estimate of  $\Pi$ , which it is as well to avoid, especially when the number of individuals is not large.  $\Pi$  is, of course, zero in the theory of two factors. By contrast with this, Spearman's tetrad difference  $F$  has a more complicated distribution. No one yet knows what its mean value in samples is, although Pearson has approximated to it (appendix).

To proceed, the standard deviation of  $P$  in samples is next obtained (see appendix). It is given in terms of quantities  $\alpha_{pq}$ , the co-variances, corresponding to  $a_{pq}$ , in the sampled population. We have, however, no means of knowing what these quantities are, and must be content with substituting the sample values, *i.e.* the  $a$ 's. The error of such substitution is not to be taken as a special defect of the present exposition, for it exists in many other fields of enquiry. Our result is as follows: let us represent by  $D$  the determinant of the fourth order of which  $a_{pq}$  is the element in the  $p$ th row and  $q$ th column. If we divide this into four equal smaller determinants by vertical and horizontal lines, calling that in the top left corner  $D_{12}$  and that in the bottom right corner  $D_{34}$ , then the standard deviation of  $P$ , when the two-factor theory holds, is given by

$$(N-2) \sigma_P^2 = \frac{N+1}{N-1} D_{12}D_{34} - D \dots\dots(3).$$

This expression is exact, except for the uncertainty of the assumption that the  $a$ 's adequately represent the  $\alpha$ 's of the sampled population. For the general case where the tetrad difference is not zero we require to add to (3) a term equal to three times the product of the other two quadrants of the determinant  $D$ , *i.e.* the top right and bottom left.

Now the significance of any observed value of  $P$  can be tested by examining the actual value reached in an experiment in the light of its standard deviation as given by (3). But a comparison is possible with data already accumulated in terms of the correlation coefficient  $r$  by writing

$$a_{pq} = s_p s_q r_{pq},$$

where  $s_p, s_q$  are the standard deviations of the  $p$ th and  $q$ th abilities in the sample. We must assume further that  $\sigma_F^2/(s_1 s_2 s_3 s_4)^2$  may be replaced by  $\sigma_F^2$ . If this is done we have

$$(N-2)\sigma_F^2 = \frac{N+1}{N-1}(1-r_{12}^2)(1-r_{34}^2) - R \quad \dots\dots(4),$$

where  $R$  is the determinant of the  $r$ 's for the four abilities. If this result be compared with the Spearman-Holzinger approximation<sup>1</sup>, two changes will be noticed: (a) The multiplier of  $\sigma_F^2$  is changed from  $N$  to  $N-2$ . (b) The term of order  $1/N$  on the right-hand side is now

$$\frac{2}{N-1}(1-r_{12}^2)(1-r_{34}^2),$$

as compared with the Spearman and Holzinger term

$$\frac{1}{N}[(1-r_{13}^2)^2(1-r_{24}^2)^2 + (1-r_{14}^2)^2(1-r_{23}^2)^2].$$

Both changes are in the direction of increasing the standard deviation of  $F$ .

Example: Holzinger's data (this *Journal*, xv, p. 18).

This case was worked out by Pearson and Moul from the full formula (*loc. cit.* pp. 272-4), every one of the 378 tetrad differences being calculated individually. If we take their figures and add in the sum of all products like  $(1-r_{12}^2)(1-r_{34}^2)$ , amounting to 6.7022, we find

$$\begin{aligned} \sigma_F^2 &= .005963, & \sigma_F &= .07722, \\ \text{'probable error' of } F &= .05208 & & \dots\dots(5). \end{aligned}$$

On the other hand, the value given by the formula of Pearson and Moul was

$$\sigma_F = .07152, \text{ 'probable error' of } F = .04824 \quad \dots\dots(6).$$

When we compare these values with the observed distribution of tetrad differences (Pearson and Moul, *loc. cit.* p. 280) we find

$$\text{observed } \sigma_F = .07708, \text{ 'probable error' of } F = .05199 \quad \dots\dots(7).$$

Thus the discrepancy of the observations from the two-factor theory, asserted to exist when (6) and (7) were compared, is seen to disappear when we replace (6) by (5). Again, if we obtain from (4) the analogue to Spearman and Holzinger's 'average' formula (this *Journal*, xvi, p. 88) we find that it yields a 'probable error' of .05200. The extraordinarily close agreement with (7) may be regarded as fortuitous, but we can at least say that in one example we have obtained striking confirmation with the two-factor theory.

<sup>1</sup> This *Journal*, xv, 1924, p. 19.

It should be noted that the 'probable error' has its customary significance only when the distribution is normal. This is certainly not the case with the tetrad. It follows that the standard deviation, while useful, requires to be supplemented by additional information, and the need will eventually arise for a table giving the values of the tetrad for various probabilities of occurrence, and based on the exact distribution.

### APPENDIX.

[Use will be made in what follows of the table of moments in *Biometrika*, **xx**, 1928, pp. 44-52.]

Let 
$$P' = a_{13}a_{24} - a_{14}a_{23},$$
 where 
$$a_{pq} = \frac{1}{N} \sum_1^N (x_p - \bar{x}_p)(x_q - \bar{x}_q).$$

#### *Mean value of $P'$ in samples.*

This is simply obtained by determining the difference between the uncorrected moments

$$\mu' \left( \begin{smallmatrix} \cdot & \cdot & 1 & \cdot \\ & \cdot & \cdot & \cdot \\ & & \cdot & \cdot \\ & & & \cdot \end{smallmatrix} \right) \text{ and } \mu' \left( \begin{smallmatrix} \cdot & \cdot & \cdot & 1 \\ & \cdot & \cdot & \cdot \\ & & \cdot & \cdot \\ & & & \cdot \end{smallmatrix} \right),$$

in the notation of the paper referred to above. The moments there given are about the mean of the distribution, and to obtain the uncorrected moments we require to make use of the relation

$$\mu_{11}' = \mu_{11} + \mu_{10}\mu_{01}.$$

Alternatively the uncorrected moments could be obtained directly from differentiation of the moment generating function (*loc. cit.* p. 41). In either case we find

$$\begin{aligned} \mu' \left( \begin{smallmatrix} \cdot & \cdot & 1 & \cdot \\ & \cdot & \cdot & \cdot \\ & & \cdot & \cdot \\ & & & \cdot \end{smallmatrix} \right) &= \frac{N-1}{N^2} \sigma_1 \sigma_2 \sigma_3 \sigma_4 [\rho_{12}\rho_{34} + \rho_{14}\rho_{23} + (N-1)\rho_{13}\rho_{24}], \\ \mu' \left( \begin{smallmatrix} \cdot & \cdot & \cdot & 1 \\ & \cdot & \cdot & \cdot \\ & & \cdot & \cdot \\ & & & \cdot \end{smallmatrix} \right) &= \frac{N-1}{N^2} \sigma_1 \sigma_2 \sigma_3 \sigma_4 [\rho_{12}\rho_{34} + \rho_{13}\rho_{24} + (N-1)\rho_{14}\rho_{23}]. \end{aligned}$$

In these expressions the  $\sigma$ 's and  $\rho$ 's are the values in the sampled population.

It follows that  $\bar{P}'$ , the mean value of  $P'$  in samples, is equal to

$$\frac{(N-1)(N-2)}{N^2} \sigma_1 \sigma_2 \sigma_3 \sigma_4 (\rho_{13}\rho_{24} - \rho_{14}\rho_{23}),$$

which may also be written

$$\frac{(N-1)(N-2)}{N^2} (\alpha_{13}\alpha_{24} - \alpha_{14}\alpha_{23}),$$

where  $\alpha$  stands for the co-variance in the population from which the sample has been taken.

*Variance of  $P'$  in samples.*

We have

$$P'^2 = a_{13}^2 a_{24}^2 + a_{14}^2 a_{23}^2 - 2a_{13}a_{14}a_{23}a_{24},$$

and so for the second moment coefficient of  $P'$  we require the values of the uncorrected moments

$$\mu' \begin{pmatrix} \cdot & \cdot & 2 & \cdot \\ \cdot & \cdot & 2 & \cdot \\ \cdot & \cdot & \cdot & \cdot \end{pmatrix}, \quad \mu' \begin{pmatrix} \cdot & \cdot & \cdot & 2 \\ \cdot & \cdot & \cdot & 2 \\ \cdot & \cdot & \cdot & \cdot \end{pmatrix}, \quad \mu' \begin{pmatrix} \cdot & \cdot & 11 \\ \cdot & \cdot & 11 \\ \cdot & \cdot & \cdot \end{pmatrix}.$$

The corresponding corrected moments are found directly from the table (*loc. cit.* p. 48), and they are converted, using the relations

$$\begin{aligned} \mu_{22}' &= \mu_{22} + 2\mu_{21}\mu_{01} + 2\mu_{12}\mu_{10} + \mu_{20}\mu_{01}^2 + \mu_{02}\mu_{10}^2 + 4\mu_{11}\mu_{10}\mu_{01} + \mu_{10}^2\mu_{01}^2, \\ \mu_{1111}' &= \mu_{1111} + \mu_{1110}\mu_{0001} + \mu_{1101}\mu_{0010} + \mu_{1011}\mu_{0100} + \mu_{0111}\mu_{1000} + \mu_{1100}\mu_{0010}\mu_{0001} \\ &\quad + \mu_{1010}\mu_{0100}\mu_{0001} + \mu_{1001}\mu_{0100}\mu_{0010} + \mu_{0110}\mu_{1000}\mu_{0001} + \mu_{0101}\mu_{1000}\mu_{0010} \\ &\quad + \mu_{0011}\mu_{1000}\mu_{0100} + \mu_{1000}\mu_{0100}\mu_{0010}\mu_{0001}. \end{aligned}$$

We have in succession

$$\begin{aligned} \mu' \begin{pmatrix} \cdot & \cdot & 2 & \cdot \\ \cdot & \cdot & 2 & \cdot \\ \cdot & \cdot & \cdot & \cdot \end{pmatrix} &= \frac{N-1}{N^4} \sigma_1^2 \sigma_2^2 \sigma_3^2 \sigma_4^2 [(N-1)(1+N\rho_{13}^2)(1+N\rho_{24}^2) \\ &\quad + 2(\rho_{12}^2 + \rho_{14}^2 + \rho_{23}^2 + \rho_{34}^2) + 2N(\rho_{12}^2 \rho_{34}^2 + \rho_{14}^2 \rho_{23}^2) \\ &\quad + 4N(\rho_{12}\rho_{13}\rho_{23} + \rho_{12}\rho_{14}\rho_{24} + \rho_{13}\rho_{14}\rho_{34} + \rho_{23}\rho_{24}\rho_{34}) \\ &\quad + 4N^2 \rho_{13}\rho_{24}(\rho_{12}\rho_{34} + \rho_{14}\rho_{23}) + 4(N+2)\rho_{12}\rho_{14}\rho_{23}\rho_{34}] \\ &\quad \dots\dots(8). \end{aligned}$$

$$\mu' \begin{pmatrix} \cdot & \cdot & \cdot & 2 \\ \cdot & \cdot & \cdot & 2 \\ \cdot & \cdot & \cdot & \cdot \end{pmatrix} \text{ is similar, } \dots\dots(9),$$

and is obtained from (8) by interchanging the suffixes 3 and 4.

$$\begin{aligned} \mu' \begin{pmatrix} \cdot & \cdot & 11 \\ \cdot & \cdot & 11 \\ \cdot & \cdot & \cdot \end{pmatrix} &= \frac{N-1}{N^4} \sigma_1^2 \sigma_2^2 \sigma_3^2 \sigma_4^2 [1 + N(\rho_{12}^2 + \rho_{13}^2 + \rho_{14}^2 + \rho_{23}^2 + \rho_{24}^2 + \rho_{34}^2) \\ &\quad + (N+2)\rho_{12}^2 \rho_{34}^2 + N^2(\rho_{13}^2 \rho_{24}^2 + \rho_{14}^2 \rho_{23}^2) \\ &\quad + (N^2 + N + 2)(\rho_{12}\rho_{13}\rho_{23} + \rho_{12}\rho_{14}\rho_{24} + \rho_{13}\rho_{14}\rho_{34} + \rho_{23}\rho_{24}\rho_{34}) \\ &\quad + (N^2 + 5N + 2)\rho_{12}\rho_{34}(\rho_{13}\rho_{24} + \rho_{14}\rho_{23}) \\ &\quad + N(N^2 + N + 2)\rho_{13}\rho_{14}\rho_{23}\rho_{34}] \\ &\quad \dots\dots(10). \end{aligned}$$

Adding (8) and (9) and subtracting twice (10) we get for the uncorrected second moment of  $P'$  the expression

$$\begin{aligned} \mu_2'(P') = & \frac{(N-1)(N-2)}{N^4} \sigma_1^2 \sigma_2^2 \sigma_3^2 \sigma_4^2 [2(1-\rho_{12}^2)(1-\rho_{34}^2) \\ & + (N-1)\{\rho_{13}^2 + \rho_{14}^2 + \rho_{23}^2 + \rho_{24}^2 \\ & - 2(\rho_{12}\rho_{13}\rho_{23} + \rho_{12}\rho_{14}\rho_{24} + \rho_{13}\rho_{14}\rho_{34} + \rho_{23}\rho_{24}\rho_{34}) \\ & + 2\rho_{12}\rho_{34}(\rho_{13}\rho_{24} + \rho_{14}\rho_{23}) + N(\rho_{13}\rho_{24} - \rho_{14}\rho_{23})^2\}]. \end{aligned}$$

Finally we have

$$\mu_2(P') = \mu_2'(P') - \{\mu_1'(P')\}^2 = \mu_2'(P') - (\bar{P}')^2,$$

giving variance of  $P'$  in samples =  $\sigma_{P'}^2$

$$\begin{aligned} = & \frac{(N-1)(N-2)}{N^4} \sigma_1^2 \sigma_2^2 \sigma_3^2 \sigma_4^2 [2(1-\rho_{12}^2)(1-\rho_{34}^2) \\ & + (N-1)\{\rho_{13}^2 + \rho_{14}^2 + \rho_{23}^2 + \rho_{24}^2 \\ & - 2(\rho_{12}\rho_{13}\rho_{23} + \rho_{12}\rho_{14}\rho_{24} + \rho_{13}\rho_{14}\rho_{34} + \rho_{23}\rho_{24}\rho_{34}) \\ & + 2\rho_{12}\rho_{34}(\rho_{13}\rho_{24} + \rho_{14}\rho_{23}) + 2(\rho_{13}\rho_{24} - \rho_{14}\rho_{23})^2\}] \dots\dots(11). \end{aligned}$$

If now we alter our definition<sup>1</sup>, as explained on p. 182, so that

$$P = \frac{1}{(N-1)(N-2)} \left[ \frac{1}{N} S(x_1 - \bar{x}_1)(x_3 - \bar{x}_3) \frac{1}{N} S(x_2 - \bar{x}_2)(x_4 - \bar{x}_4) - \frac{1}{N} S(x_1 - \bar{x}_1)(x_4 - \bar{x}_4) \frac{1}{N} S(x_2 - \bar{x}_2)(x_3 - \bar{x}_3) \right],$$

we have, simply,

$$\bar{P} = \Pi.$$

[Compare with Pearson and Moul, *loc. cit.* p. 249, who gave

$$\bar{T} = \rho T \left( 1 + \frac{S(\rho^2) - 4}{2N} \right), \text{ approximately.}]$$

Also we have for the variance of  $P$

$$\begin{aligned} \sigma_{P'}^2 = & \frac{\sigma_1^2 \sigma_2^2 \sigma_3^2 \sigma_4^2}{N-2} \left[ \rho_{13}^2 + \rho_{14}^2 + \rho_{23}^2 + \rho_{24}^2 \right. \\ & - 2(\rho_{12}\rho_{13}\rho_{23} + \rho_{12}\rho_{14}\rho_{24} + \rho_{13}\rho_{14}\rho_{34} + \rho_{23}\rho_{24}\rho_{34}) \\ & + 2\rho_{12}\rho_{34}(\rho_{13}\rho_{24} + \rho_{14}\rho_{23}) + 2(\rho_{13}\rho_{24} - \rho_{14}\rho_{23})^2 \\ & \left. + \frac{2}{N-1}(1-\rho_{12}^2)(1-\rho_{34}^2) \right] \dots\dots(12), \end{aligned}$$

which may be written

$$(N-2)\sigma_{P'}^2 = \frac{N+1}{N-1} |a_{11}a_{22}| |a_{33}a_{44}| + 3|a_{13}a_{24}|^2 - \Delta \dots\dots(12 a),$$

where  $\Delta$  is the determinant  $|a_{pq}|$   $p, q = 1, 2, 3, 4$ .

<sup>1</sup> This is equivalent, in uni-variate problems, to defining the sampling variance as  $\frac{1}{N-1} \sum_1^N (x - \bar{x})^2$ , in order to ensure that the mean value of this quantity in samples shall be equal to the variance ( $\sigma^2$ ) of the sampled population.

If the tetrad is zero in the sampled population the second term vanishes and we get equation (3), which may also be written, in terms of  $\rho$

$$\frac{(N-2) \sigma_P^2}{\sigma_1^2 \sigma_2^2 \sigma_3^2 \sigma_4^2} = \frac{N+1}{N-1} (1 - \rho_{12}^2) (1 - \rho_{34}^2) - R', \dots\dots(12 b),$$

where  $R'$  is the determinant

$$| \rho_{pq} |, \quad p, q = 1, 2, 3, 4.$$

This leads to equation (4) of text.

One further result should be put on record here. The mean value in samples of the determinant  $D$  (see p. 182) is equal to

$$\frac{(N-1)(N-2)(N-3)(N-4)}{N^4} \Delta,$$

and, more generally, to

$$\frac{(N-1)(N-2) \dots (N-p)}{N^p} \Delta$$

for a determinant of the  $p$ th order, or any minor of this order from a larger determinant. This is the first step in the determination of the probable error of  $\sigma_P^2$ .

*(Manuscript received 11 April, 1928.)*



ON THE FREQUENCY DISTRIBUTION OF ANY NUMBER OF DEVIATES FROM THE MEAN OF A SAMPLE FROM A NORMAL POPULATION AND THE PARTIAL CORRELATIONS BETWEEN THEM.

By J. O. IRWIN, M.A., M.Sc. (Rothamsted Experimental Station).

LET  $x_1, x_2, \dots, x_n$  be a set of sample values from a normal population whose mean is  $m$  and standard deviation  $\sigma$ , the probability of obtaining such a sample is

$$Ce^{-\frac{1}{2} \frac{S(x_r - m)^2}{\sigma^2}} dx_1 dx_2 \dots dx_n \quad \dots \quad (1)$$

$$\text{Since} \quad S(x_r - m)^2 = S(x_r - \bar{x})^2 + n(\bar{x} - m)^2$$

we may write (1)

$$Ce^{-\frac{n(\bar{x} - m)^2}{2\sigma^2}} e^{-\frac{1}{2} \frac{S(x_r - \bar{x})^2}{\sigma^2}} dx_1 dx_2 \dots dx_n \quad \dots \quad (2)$$

Let us now transform (2) by writing

$$\begin{aligned} x_1 &= \bar{x} + X_1 \\ x_2 &= \bar{x} + X_2 \\ &\dots \dots \dots \\ x_{n-1} &= \bar{x} + X_{n-1} \\ x_n &= \bar{x} + X_n = \bar{x} - X_1 - X_2 \dots \dots - X_{n-1} \end{aligned} \quad \dots \quad (3)$$

The expression (2) becomes

$$C' e^{-\frac{n(\bar{x} - m)^2}{2\sigma^2}} dx e^{-\frac{1}{2} \left\{ \sum_{r=1}^{n-1} \frac{X_r^2}{\sigma^2} + \frac{(X_1 + X_2 + \dots + X_{n-1})^2}{\sigma^2} \right\}} dX_1 dX_2 \dots dX_{n-1} \quad \dots \quad (4)$$

Now  $X_1, X_2, \dots, X_{n-1}$  are independent and are the deviations of  $(n-1)$  of the sample observations from the sample mean.

From (4) it follows that their joint distribution is

$$C'' e^{-\frac{1}{\sigma^2} (X_1^2 + X_2^2 + \dots + X_{n-1}^2 + \sum_{r,s=1}^{n-1} X_r X_s)} dX_1 dX_2 \dots dX_{n-1}.$$

By writing this

$$C'' e^{-\frac{1}{\sigma^2} \left\{ (X_{n-1} + \frac{1}{2}(X_1 + \dots + X_{n-2}))^2 + \sum_{r,s=1}^{n-2} X_r X_s - \frac{1}{4}(X_1 + \dots + X_{n-2})^2 \right\}} dX_1 dX_2 \dots dX_{n-1}$$

and by integrating out for  $X_{n-1}$  we find for the simultaneous distribution of  $X_1, X_2, \dots, X_{n-2}$

$$\text{const. } e^{-\frac{1}{\sigma^2}\{a_1(X_1^2 + \dots + X_{n-2}^2) + \frac{1}{2} \sum_{r,s=1}^{n-2} X_r X_s\}} dX_1 \dots dX_{n-2} \quad (5)$$

The process may easily be generalized so as to obtain the joint distribution of  $X_1, X_2, \dots, X_{n-r}$

For if we consider the distribution

$$\text{const. } e^{-\frac{1}{\sigma^2}\{a_1(X_1^2 + X_2^2 + \dots + X_{n-1}^2) + b_1 \sum_{r,s=1}^{n-1} X_r X_s\}} dX_1 \dots dX_{n-1}$$

the elimination of  $X_{n-1}$  gives for the distribution of the remainder

$$\text{const. } e^{-\frac{1}{\sigma^2}\{a_2(X_1^2 + \dots + X_{n-2}^2) + b_2 \sum_{r,s=1}^{n-2} X_r X_s\}} dX_1 \dots dX_{n-2}$$

$$\text{where} \quad a_2 = a_1 - \frac{b_1^2}{4a_1} \quad b_2 = b_1 - \frac{b_1^2}{2a_1}$$

whence in general

$$a_r = a_{r-1} - \frac{b_{r-1}^2}{4a_{r-1}} \quad b_r = b_{r-1} - \frac{b_{r-1}^2}{2a_{r-1}}$$

$$\text{or} \quad a_r - \frac{1}{2}b_r = a_{r-1} - \frac{1}{2}b_{r-1} = \dots = a_1 - \frac{1}{2}b_1.$$

Now in this case  $a_1 = b_1 = 1$ .

$$\text{Thus} \quad a_r = \frac{1}{2}(b_r + 1) \\ b_r = b_{r-1} - \frac{b_{r-1}^2}{b_{r-1} + 1} = \frac{b_{r-1}}{b_{r-1} + 1}.$$

$$\text{Thus} \quad \frac{1}{b_r} = 1 + \frac{1}{b_{r-1}} = r - 1 + \frac{1}{b_1} = r$$

$$\text{or} \quad \left. \begin{aligned} b_r &= \frac{1}{r} \\ a_r &= \frac{r+1}{2r} \end{aligned} \right\} \dots \dots \dots (6)$$

So the elimination of  $X_{n-r+1}, \dots, X_{n-1}$  leaves for the joint distribution of  $X_1, X_2, \dots, X_{n-r}$

$$K e^{-\frac{1}{\sigma^2}\left\{\frac{r+1}{2r}(X_1^2 + \dots + X_{n-r}^2) + \frac{1}{r} \sum_{u,v=1}^{n-r} X_u X_v\right\}} dX_1 \dots dX_{n-r} \quad (7)$$

This is the joint distribution of any  $(n-r)$  deviates from the mean of the sample.

As particular cases we have for the joint distribution of  $X_1$  and  $X_2$

$$\text{const. } e^{-\frac{1}{\sigma^2}\left\{\frac{n-1}{2(n-2)}(X_1^2 + X_2^2) + \frac{1}{n-2}X_1X_2\right\}} dX_1 dX_2 \quad (8)$$

and for the distribution of  $X_1$

$$\text{const. } e^{-\frac{nX_1^2}{2(n-1)\sigma^2}} dX_1 \quad (9)$$

(9) is a classical result and (8) has been given by R. A. Fisher,\* and the well-known results

$$\left. \begin{aligned} \sigma_{X_u} &= \sigma_{X_1} = \frac{(n-1)\sigma^2}{n} \\ r_{X_u X_v} &= r_{X_1 X_2} = -\frac{1}{n-1} \end{aligned} \right\} \dots \dots (10)$$

are at once deduced.

From (7) we may deduce the partial correlation between any two deviates when  $n-r-2$  of the remaining  $n-2$  are kept constant.

For the general normal surface for  $n-r$  variables  $X_1, X_2, \dots, X_{n-r}$  referred to their means as origin is given by

$$Z = Z_0 e^{-\frac{1}{2\Delta} \left\{ \sum_{s=1}^{n-1} \frac{\Delta_{ss} X_s^2}{\sigma_s^2} + 2 \sum_{u,v=1}^{n-r} \Delta_{uv} \frac{X_u X_v}{\sigma_u \sigma_v} \right\}} \dots (11)$$

where

$$Z_0 = \frac{1}{(\sqrt{2\pi})^{n-r} \sigma_1 \sigma_2 \dots \sigma_{n-r} \sqrt{\Delta}}$$

$$\Delta = \begin{vmatrix} 1 & r_{12} & \dots & r_{1,n-r} \\ r_{21} & 1 & \dots & r_{2,n-r} \\ \vdots & \vdots & \ddots & \vdots \\ r_{n-r,1} & r_{n-r,2} & \dots & 1 \end{vmatrix},$$

$r_{uv}$  is the correlation between  $X_u$  and  $X_v$  and  $\Delta_{uv}$  is the prepared minor of  $r_{uv}$  in  $\Delta$ .

By comparison with (7) we have

$$\left. \begin{aligned} \frac{\Delta_{ss}}{2\Delta\sigma_s^2} &= \frac{r+1}{2r\sigma^2} \\ \frac{\Delta_{ur}}{\Delta\sigma_u\sigma_v} &= \frac{1}{r\sigma^2} \end{aligned} \right\}$$

or

$$\left. \begin{aligned} \frac{\Delta_{ss}}{\Delta} &= \frac{(r+1)(n-1)}{nr} \\ \frac{\Delta_{ur}}{\Delta} &= \frac{n-1}{nr} \end{aligned} \right\} \dots (12)$$

But the partial correlation between  $X_u$  and  $X_r$ , the remaining  $n-r-2$  deviates being constant, is given by

$$\rho_{ur, 12 \dots (n-r)} = -\frac{\Delta_{ur}}{\sqrt{\Delta_{uu}\Delta_{rr}}}.$$

Hence

$$\rho_{ur, 12 \dots (n-r)} = -\frac{1}{(r+1)}.$$

\* R. A. Fisher, *Monthly Notices of the Royal Astronomical Society*, Vol. LXXX, No. 8, p. 761.

If we take  $r = 0$  we obtain

$$\rho_{uv, 12 \dots (n)} = -1$$

or the correlation between two deviates when *all* the remaining deviates are kept constant is negative and perfect, as is otherwise evident.

The discussion may be completed by determining the constant  $K$  in (7).

We have

$$K = \frac{1}{(\sqrt{2\pi})^n \sigma_1 \sigma_2 \dots \sigma_{n-r} \sqrt{\Delta}}$$

where

$$\sigma_1 = \sigma_2 = \dots = \sigma_{n-r} = \sqrt{\frac{n-1}{n}} \sigma$$

and  $\Delta$  is the  $n - r^{\text{th}}$  order determinant

$$\begin{vmatrix} 1 & -\frac{1}{n-1} & -\frac{1}{n-1} & \dots & -\frac{1}{n-1} \\ -\frac{1}{n-1} & 1 & -\frac{1}{n-1} & \dots & -\frac{1}{n-1} \\ \cdot & \cdot & \cdot & \cdot & \cdot \\ -\frac{1}{n-1} & -\frac{1}{n-1} & -\frac{1}{n-1} & \dots & 1 \end{vmatrix}$$

Now it is easily shown that the  $n^{\text{th}}$  order determinant

$$\begin{vmatrix} 1 & \epsilon & \epsilon & \epsilon & \dots & \epsilon \\ \epsilon & 1 & \epsilon & \epsilon & \dots & \epsilon \\ \epsilon & \epsilon & 1 & \epsilon & \dots & \epsilon \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \epsilon & \epsilon & \epsilon & \epsilon & \dots & 1 \end{vmatrix} = (1 - \epsilon)^{n-1} (1 + \overline{n-1}\epsilon).$$

$$\begin{aligned} \text{Hence } \Delta &= \left(1 + \frac{1}{n-1}\right)^{n-r-1} \left(1 - \frac{n-r-1}{n-1}\right) \\ &= \left(\frac{n}{n-1}\right)^{n-r-1} \left(\frac{r}{n-1}\right) \end{aligned}$$

$$\text{and } \sigma_1 \sigma_2 \dots \sigma_{n-r} \sqrt{\Delta} = \sqrt{\frac{n-1}{n}} \sqrt{\frac{r}{n-1}} \sigma^{n-r} = \sqrt{\frac{r}{n}} \sigma^{n-r},$$

$$\text{and } K = \frac{1}{(\sqrt{2\pi}\sigma)^{n-r} \sqrt{\frac{r}{n}}}.$$

*Summary.*

(1) The joint frequency distribution of any  $(n - r)$  deviates from the mean of a sample from a normal population is given by

$$df = \frac{1}{(\sqrt{2\pi}\sigma)^{n-r}} \sqrt{\frac{n}{r}} e^{-\frac{1}{2\sigma^2} \left\{ \frac{r+1}{2r} (X_1^2 + \dots + X_{n-r}^2) + \frac{1}{r} \sum_{u,v=1}^{n-r} X_u X_v \right\}} dX_1 \dots dX_{n-r}.$$

(2) The partial correlation coefficient between any two of these  $(n - r)$  deviates, the remaining  $(n - r - 2)$  being kept constant,

$$\text{is } -\frac{1}{(r+1)}.$$


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NOTE ON THE  $\chi^2$  TEST FOR GOODNESS  
OF FIT

# NOTE ON THE $\chi^2$ TEST FOR GOODNESS OF FIT.

By J. O. IRWIN, M.A., M.Sc.

(Rothamsted Experimental Station.)

SUPPOSE we have a set of observed frequencies  $n_1, n_2 \dots n_s$  (total  $N$ ) and that by some process we have fitted a curve to them, the "theoretical" frequencies being  $m_1, m_2 \dots m_s$  (total  $N$ ), and that we wish to test for goodness of fit. There are two alternative hypotheses which we may regard ourselves as testing.

I. We may suppose that we have a hypothetically infinite population in which the proportional frequencies in the  $s$  categories are  $p_1, p_2 \dots p_s$

$$\text{where } p_r = \frac{m_r}{N},$$

and that samples of  $N$  are drawn at random from this population, and that for each sample

$$\chi^2 = S \left\{ \frac{(m_r - n_r)^2}{m_r} \right\} \text{ is calculated.}$$

Here  $m_1, m_2 \dots m_s$  are taken as the population values and are therefore supposed to be invariable from sample to sample. In this case it may be shown that the distribution of  $\chi^2$  is

$$C(\chi^2)^{\frac{s-3}{2}} e^{-\frac{1}{2}\chi^2} d\chi^2.$$

This is the Classical Pearsonian Method.

II. We may suppose our proportional frequencies in the population to be  $\frac{M_1}{N}, \frac{M_2}{N}, \dots \frac{M_s}{N}$  where we do not know the values of the  $M$ 's, and that samples of  $N$  are drawn at random from it, and that from the observed frequencies  $n_1, n_2 \dots n_s$  in each sample a set of theoretical frequencies  $m_1', m_2' \dots m_s'$  are estimated by the same process as is adopted in the actually observed sample, and that for each sample

$$\chi^2 = S \left\{ \frac{(m_r' - n_r)^2}{m_r'} \right\} \text{ is calculated.}$$

Here  $m_1', m_2' \dots m_s'$  vary from sample to sample, and in the observed sample  $m_r' = m_r$ .

In this case the distribution of  $\chi^2$  will depend on the process of

fitting employed; if four moments are used in fitting, then it may be shown that the distribution of  $\chi^2$  is

$$C(\chi^2)^{\frac{s-7}{2}} e^{-\frac{1}{2}\chi^2} d\chi^2,$$

provided the statistical estimates used are efficient.

The two results differ because we are calculating the frequency distributions of two different quantities, and it is not a question for mathematics alone to determine which method is the more reasonable.

The writer favours the second method, first, because it dispenses with the somewhat arbitrary assumption of a particular population

$\frac{m_1}{N}, \frac{m_2}{N} \dots \frac{m_s}{N}$  from which to test divergence—a population which

naturally is not quite coincident with the true population; secondly, because it assumes that  $\chi^2$  is calculated from each sample by the method actually used in the observed sample and makes no arbitrary assumption about the population frequencies at all. Of course we are at liberty to test divergence from any population we please, and in particular from one in which the proportional frequencies are

$\frac{m_1}{N} \dots \frac{m_r}{N} \dots \frac{m_s}{N}$ ; it is only a question whether it is appropriate to

do so. It seems to the writer inappropriate because, if we do so, we are selecting a population, from which to test, which is biased in the direction of the observed sample, and the resulting value of  $\chi^2$  is consequently too small or we exaggerate the probability that the sample was drawn from the population. The reader can realize this very easily if he remembers that, if we had chosen a curve for fitting with enough disposable constants in it, every  $m$  could have been made equal to the corresponding  $n$ , and  $\chi^2$  would then have been zero.

Experimental evidence is not in itself conclusive, as an experiment may be designed to test either hypothesis. For example, if we tossed 10 pennies 100 times and recorded the frequencies of 0, 1, 2 . . . 10 heads and repeated the process a large number of times, say 1,000, if we calculated our theoretical frequencies from the binomial  $100(\frac{1}{2} + \frac{1}{2})^{10}$  in each case, then we should verify that  $\chi^2$  was distributed as

$$C(\chi^2)^{\frac{s-3}{2}} e^{-\frac{1}{2}\chi^2} d\chi^2,$$

$s$  being in this case 11.

But if we assume our binomial to be  $100(p + q)^{10}$  and estimate  $p$  (and  $q$ ) separately for each sample by the usual method of equating the mean of the sample to the corresponding quantity in the binomial, subsequently calculating the theoretical frequencies from the values of  $p$  and  $q$  so determined, and finally calculate  $\chi^2$  for each

sample, using these "theoretical frequencies," then we should verify that  $\chi^2$  was distributed as

$$C(\chi^2)^{\frac{s-4}{2}} e^{-\frac{1}{2}\chi^2} d\chi^2.$$

The reviewer of Mr. Fry's *Probability* \* in the *Journal of the Royal Statistical Society* remarks that methods of fitting may be adopted for which we should not know the distribution of  $\chi^2$  on the second hypothesis. The writer agrees that such methods may be adopted, but thinks they do not test any interesting hypothesis. For example, to take the reviewer's case, by applying the goodness of fit test to a curve drawn by eye by an expert draughtsman we can only test the ingenuity of the draughtsman in making the frequencies of the fitted curve agree as nearly as possible with those of the histogram.

In any case the existence of such methods is no argument for not using hypothesis II whenever possible, as it is the least artificial one that can be made. It must, however, always be borne in mind that mathematics alone cannot decide the question. Only when we have decided the precise question we wish to ask will mathematics provide the answer.

These remarks have been made after a careful perusal of the available literature on both sides of the question, and it is hoped they may be of use to readers of the *Journal of the Royal Statistical Society*.

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## A STATISTICAL ANALYSIS OF THE DAILY OBSERVATIONS OF THE MAXIMUM AND MINIMUM THERMOMETERS AT ROTHAMSTED.

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The meteorological records at Rothamsted Experimental Station are possibly among the oldest in this country, accurate observations having now been taken for very nearly fifty years. Over this period a vast quantity of data has been accumulated, and it has been the task of the Statistical Department to sort out and make use of this somewhat unwieldy material.

The records of rainfall and sunshine have already been dealt with, and it has been the privilege of the present writer, during his stay at Rothamsted, to investigate those relating to maximum and minimum temperature.

These records go back to September 1878, and consist of daily readings of the maximum and minimum thermometers in degrees Fahrenheit over this period.

### OBJECT OF INVESTIGATION.

Temperature is a very important factor in the life of an agricultural crop, for although in this country it may not be the most important factor, except in extreme cases, in any particular season a departure from the normal behaviour of the temperature may influence a crop materially. Thus, in May the occurrence of frosts is not normal at this station; frosts, however, do occur, and when they do, as they did this year, the damage to the blossoms of fruit trees may be of considerable economic importance.

It was therefore considered necessary, before considering the influence of the variations of temperature on any particular agricultural or horticultural crop, to determine the normal behaviour of temperature in this district. With the large number of observations to be considered, it seemed possible to determine not only the actual mean temperature at any one time of year, but also the degree to which that temperature might be expected to vary from the normal at that season and its influence on any crop under consideration.

At the same time it was recognized that the daily temperature records at meteorological stations could not readily be utilized without a comprehensive reduction of the data which would bring out the salient characteristics of the temperature experienced in that district.

The method utilized by the writer is therefore given in some detail in the hope that other stations may adopt some similar method to analyze their existing temperature records. In the future a repetition of this somewhat laborious process can be avoided by carrying out this reduction as a routine when the monthly summaries are prepared. If this is done, the investigator will be able to ascertain at a glance whether any important departure from the normal incidence of temperature in his district is evident during the period in which he is interested, without the

loss of time entailed by a detailed statistical analysis of the temperature records.

#### METHOD OF APPROACH.

The first step in this investigation was to put the data into such a form that it could be easily handled. It was considered that the smallest unit which could be conveniently dealt with was one calendar month, and accordingly for each month five statistics were obtained.

These were (1) and (2) the mean of the daily maximum and minimum temperatures, (3) and (4) the variances or mean square deviations of the above, (5) the product moment of the two.

Numbers (1) and (2) have always been found and are naturally essential to any picture of the temperature during the month.

The "variance" is simply the mean of the squared deviations from the mean monthly temperature. It represents the amount of variation in the subject under consideration during that period. From it the standard deviation and the standard error of the mean can be directly calculated.

The product moment represents the association between the two measures. It is the mean of the products of deviations of any pair of observations from their means. From it, in conjunction with the two variances, can be calculated the coefficient of correlation and any further statistics which may be required.

Let  $x_1, x_2, \dots, x_n$  and  $y_1, y_2, \dots, y_n$  represent corresponding observations of the maximum and minimum temperatures for any one month.

$$\text{Then, the monthly mean} = \bar{x} = \frac{S(x)}{n} \quad \text{and} \quad \bar{y} = \frac{S(y)}{n}$$

$$\text{Variance} = \sigma_x^2 = \frac{S(x-x)^2}{n-1} \quad \text{and} \quad \sigma_y^2 = \frac{S(y-y)^2}{n-1}$$

$$\text{Product Moment} = \frac{S(x-\bar{x})(y-\bar{y})}{n-1}$$

$$\text{Thence Standard Error of Mean} = \frac{\sigma_x}{\sqrt{n}} \quad \text{and} \quad \frac{\sigma_y}{\sqrt{n}}$$

$$\text{and the coefficient of correlation } r = \frac{\text{Product Moment}}{\sigma_x \sigma_y}$$

The following method was used in order to obtain the above statistics, and to shorten the work, one degree Fahrenheit was selected as a suitable group interval.

Table I. demonstrates how the data was set out. This table shows in a grouped form the observations of maximum temperature for the year September 1886 to August 1887 inclusive. Column 1 gives the complete range of temperature during the year in degrees Fahrenheit. Columns 2 to 13 show the frequency with which each temperature occurred. The original readings were taken to one place of decimals. For example, in September there was one day on which the maximum temperature was between  $53^{\circ}.95$  F. and  $54^{\circ}.95$  F., one day on which it was between  $54^{\circ}.95$  F. and  $55^{\circ}.95$  F. and so on. From these tables the mean and variance for each

TABLE I.—FREQUENCY OF MAXIMUM TEMPERATURES. SEPTEMBER 1886  
TO AUGUST 1887.

* F.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.
29	...	...	...	1	...	...	...	...	...	...	...	...
30	...	...	...	2	...	...	...	...	...	...	...	...
31	...	...	...	1	1	...	...	...	...	...	...	...
32	...	...	...	1	1	...	...	...	...	...	...	...
33	...	...	...	1	2	1	...	...	...	...	...	...
34	...	...	...	2	2	...	1	...	...	...	...	...
35	...	...	...	1	2	1	1	...	...	...	...	...
36	...	...	...	2	1	...	1	...	...	...	...	...
37	...	...	...	4	...	...	...	...	...	...	...	...
38	...	...	...	2	1	4	6	...	...	...	...	...
39	...	...	...	1	2	2	1	...	...	...	...	...
40	...	...	...	6	2	2	2	...	...	...	...	...
41	...	...	...	1	1	1	...	...	...	...	...	...
42	...	...	...	2	1	1	3	1	...	...	...	...
43	...	...	...	2	1	1	...	1	...	...	...	...
44	...	...	1	2	...	2	...	...	...	...	...	...
45	...	...	9	3	1	1	2	3	...	...	...	...
46	...	...	...	1	1	1	1	1	...	...	...	...
47	...	1	5	...	2	1	...	1	1	...	...	...
48	...	...	2	...	...	1	1	1	...	...	...	...
49	...	2	4	1	3	2	2	2	...	...	...	...
50	...	1	1	1	1	2	2	6	3	...	...	...
51	...	...	...	...	...	4	4	...	2	...	...	...
52	...	2	3	1	...	...	1	1	2	...	...	...
53	...	2	3	1	...	...	2	3	4	1	...	...
54	1	3	1	...	...	1	...	1	1	1	...	...
55	1	1	...	...	...	...	...	1	1	...	...	...
56	...	3	...	...	...	...	...	...	...	...	...	...
57	1	3	1	...	...	...	1	1	5	...	...	...
58	...	1	...	...	...	...	...	1	1	2	...	...
59	1	4	...	...	...	...	...	2	5	1	...	...
60	4	1	...	...	...	...	...	1	1	...	...	...
61	1	...	...	...	...	...	...	...	...	...	...	...
62	2	1	...	...	...	...	...	2	1	...	...	1
63	2	...	...	...	...	...	...	1	1	...	...	1
64	1	1	...	...	...	...	...	...	...	...	...	4
65	2	1	...	...	...	...	...	...	2	2	...	1
66	2	1	...	...	...	...	...	...	...	2	...	...
67	2	...	...	...	...	...	...	...	...	...	1	1
68	2	...	...	...	...	...	...	...	2	1	1	1
69	1	...	...	...	...	...	...	1	5	...	3	3
70	3	...	...	...	...	...	...	...	2	1	2	2
71	...	...	...	...	...	...	...	...	2	2	1	1
72	...	...	...	...	...	...	...	...	1	4	2	2
73	1	...	...	...	...	...	...	...	...	5	3	3
74	...	...	...	...	...	...	...	...	1	1	4	1
75	2	...	...	...	...	...	...	...	...	...	1	1
76	...	3	...	...	...	...	...	...	...	3	4	2
77	...	...	...	...	...	...	...	...	...	2	...	...
78	...	...	...	...	...	...	...	...	2	2	...	...
79	...	...	...	...	...	...	...	...	1	1	2	...
80	1	...	...	...	...	...	...	...	1	...	...	...
81	...	...	...	...	...	...	...	...	...	3	1	...
82	...	...	...	...	...	...	...	...	1	1	...	...
83	...	...	...	...	...	...	...	...	...	1	...	...
84	...	...	...	...	...	...	...	...	...	...	1	...
85	...	...	...	...	...	...	...	...	...	1	...	...
86	...	...	...	...	...	...	...	...	...	1	...	...
Mean	65.58	58.52	48.85	41.29	39.64	44.52	44.93	51.42	56.77	69.72	76.13	71.68
Variance	38.97	54.30	11.37	33.15	36.01	31.70	40.72	33.48	25.55	53.78	22.52	29.83

month may be worked out in the ordinary way, Sheppard's correction for grouping being used. Actually, it was not necessary to work out the variance every time, only the sum of the squared deviations from the mean being necessary for the final estimate of the normal monthly variance over a number of years. A similar method was used to find the variance of the minimum temperatures.

The product moment is found from a diagram of the type of Fig. 1. In this case the maximum temperature is plotted against

JUNE 1887.

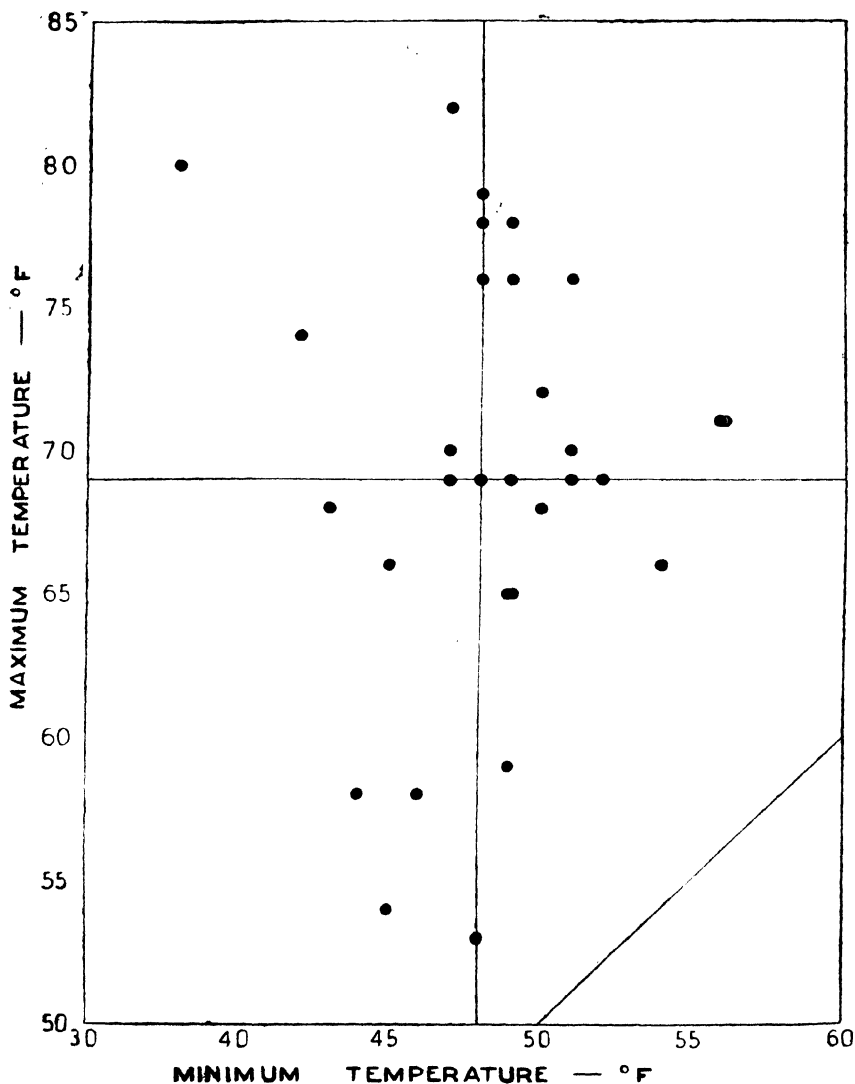


FIG. 1.—Correlation of maximum and minimum temperatures.

the minimum and from these the product terms  $(x - \bar{x})(y - \bar{y})$  may easily be read off and summed.

Diagrams of this kind also provide a very good picture of the type of weather prevalent during the month. The one selected shows a typical summer month. This particular June it will be noted was much more variable in its maximum temperature than in its minimum and the correlation was low, the points being scattered round the means, with no very apparent connection between them.

Such a diagram for January, however, would have shown that the minimum temperature was more variable than the maximum; but that there was a much greater tendency for a high maximum temperature to be accompanied by a high minimum.

NOTE.—Another and perhaps shorter method of finding the above constants, where the data are not grouped, may be used where a calculating machine is available. In this method the following formulæ are made use of:

$$S(x - \bar{x})^2 = S(x^2) - \bar{x} \cdot S(x)$$

$$S(y - \bar{y})^2 = S(y^2) - \bar{y} \cdot S(y)$$

$$S(x - \bar{x})(y - \bar{y}) = S(xy) - \bar{x} \cdot S(y)$$

It will be recognised that the reduction of the data to the above constants gives an excellent summary of each month's temperature, and is a good basis for further analysis of the data to be made, and it would be an exceedingly useful addition to meteorological records, if the variance and product moment could be added to the means when monthly summaries are being prepared.

The above methods enable the amount of variation from day to day within each month in any one year to be determined, and from this can be found the average variance of a month from day to day over a number of years.

When, however, the behaviour of, for example, October as a whole is to be considered, there is another source of variation, that from year to year, to be taken into account. If this be found, then, not only can the normal behaviour of October from day to day within a year be examined, but also it can be seen whether as a rule one October differs from another or not. It may also be understood that not only are maximum and minimum temperatures correlated from day to day in any October, but also, that from October to October there is a correlation between the maximum and minimum temperatures which is independent of the day to day correlation.

Thus, it is apparent that when the forty-nine Octobers are considered together, there is a total variance and a total correlation which is made up of two parts, that due to variation between days of individual months (the "within-month" variance) and that due to variation of the same month in different years or the "between-month" variance.

#### ANALYSIS OF VARIANCE.

The method used to determine this "between-month" variance and correlation was that of the *Analysis of Variance* as developed by R. A. Fisher (see "Statistical Methods for Research Workers," by R. A. Fisher. Oliver and Boyd).

It has already been seen how the daily variation within months has been worked out. That between months was obtained by finding the sum of squares of deviations of each October day in the 48 years from the general mean of all the October days, and subtracting therefrom the sum of squares of deviations of October days from the mean of their month as found above. This will give thirty-one times the sum of squares of each October mean from the general mean and this divided by forty-eight will give thirty-one times the between-year variance.

The process is better set forth as follows:—

Let  $(x_1 x_2 \dots x_k)$  be the readings of maximum temperature in any month of  $k$  days, and  $(y_1 y_2 \dots y_k)$  be the corresponding minimum temperatures.

Then

$$\bar{x}_p = \frac{S(x_p)}{k}$$

$$\text{and } \bar{y}_p = \frac{S(y_p)}{k} \text{ are the means for that month.}$$

There are  $(k-1)$  degrees of freedom in the month, i.e., in a month of  $k$  days, if the mean is already known, it is still possible to alter  $(k-1)$  observations arbitrarily without affecting the mean. Thus the variances will be:—

$$\frac{S(x - \bar{x}_p)^2}{k-1} \quad \text{and} \quad \frac{S(y - \bar{y}_p)^2}{k-1}$$

and the product moment will be

$$\frac{S(x - \bar{x}_p)(y - \bar{y}_p)}{k-1}$$

If now there are  $n$  years, and  $\bar{x}$  be the general mean,

$$S(x - \bar{x})^2 = k \sum_1^n (x_p - \bar{x})^2 + \sum_1^n S(x - \bar{x}_p)^2$$

This relationship may be presented in the form of a table:—

TABLE II.

	Degrees of Freedom.	Sum of Squares of Deviations.	Sum of Products.
Within months -	$(k-1)n$	$\sum_1^{kn} (x - \bar{x}_p)^2$	$\sum_1^{kn} (x - \bar{x}_p)(y - \bar{y}_p)$
Between months-	$n-1$	$k \sum_1^n (\bar{x}_p - \bar{x})^2$	$k \sum_1^n (\bar{x}_p - \bar{x})(\bar{y}_p - \bar{y})$
Total - -	$nk-1$	$\sum_1^{kn} (x - \bar{x})^2$	$\sum_1^{kn} (x - \bar{x})(y - \bar{y})$

Thence by dividing the sums of squares by the degrees of freedom the variance and, in a like way, the product moments can be deduced. (It may be observed that since  $k$  may vary from month to month, the absence of any reading does not affect the result.)



The total variances and product moment were worked out directly from the grouped data. The general mean and variance was worked out in the ordinary way, and in order to find the sum of the products, a correlation table was prepared for each month setting forth the whole fifteen hundred pairs of observations for each day. Such a table for October is shown (Table III.).

These tables are themselves interesting and in spite of the labour of construction are well worth preparing. In addition to the information required above, a large amount of information as to the frequency of occurrence of different combinations of temperature can be read off directly from the table.

The analysis for the month of October is given below:—

TABLE IV.—ANALYSIS OF VARIANCE, OCTOBER 1878–1926 (INCLUSIVE).  
MAXIMUM AND MINIMUM TEMPERATURES.

(a)	Degrees of Freedom.	Sum of Squares of Deviations.		Sum of Products.
		Maximum.	Minimum.	
Within the month -	1466	40001	60668	26698
Between months -	48	11505	11863	9067
Total - - -	1514	51506	72531	35765

(b)	Variance of:		Product Moment.
	Maximum.	Minimum.	
Within the month -	27.3	41.4	18.2
Between months -	239.7	247.1	188.9
Total - - -	34.0	47.9	23.6

From (b) can be directly calculated the coefficient of correlation.

Within the month	...	...	+ .542
Between months	...	...	+ .776
Total	...	...	+ .585

In a similar way looking at the data from another point of view the variances and product moments for the daily mean of the maximum and minimum temperatures and the daily range were worked out. The resulting variances and correlation coefficients are shown in Table V.

TABLE V.—ANALYSIS OF VARIANCE. OCTOBER 1878–1926 (INCLUSIVE).  
DAILY MEAN OF MAXIMUM AND MINIMUM TEMPERATURES AND DAILY RANGE.

	Variance of:		Product Moment.
	Mean.	Range.	
Within the month -	26.27	32.25	—7.04
Between months -	216.16	109.02	—3.73
Total - - -	32.29	34.68	—6.94

# CORRELATION COEFFICIENT.

Within the month	...	...	...	-.242
Between months	...	...	...	-.024
Total	...	...	...	-.207

From the above estimates of the variance of the daily readings about the monthly mean and  $k$  times the variance of the monthly means about the general mean, the significance of the between-month variance can be tested. That is to say, it is possible to determine whether there is any significant variation in the temperature of a month from year to year, or whether such variation is only such as might be attained by a process of random sampling. This is carried out by making a test of significance between the two variances, by the method laid down by R. A. Fisher.

If  $z$  is equal to half the difference of the natural logarithms of the variances, then the standard error of  $z$  is the square root of half the sum of the reciprocals of the degrees of freedom

$\sigma_z = \sqrt{\frac{1}{2} \left( \frac{1}{n_1} + \frac{1}{n_2} \right)}$ . This simple method of finding  $\sigma_z$ , while not strictly accurate, slightly overestimates the standard error, so that in this case, where the difference is always very significant, no information is lost.

As an example the significance of the difference between the within-month and between-month variances of the maximum temperatures for October is calculated in Table VI.

TABLE VI.—CALCULATION OF SIGNIFICANCE OF THE DIFFERENCE BETWEEN THE  
" WITHIN-MONTH " AND " BETWEEN-MONTH " VARIANCES.  
MAXIMUM TEMPERATURES. OCTOBER, 1878-1926.

	Degrees of Freedom.	Variance.	$\frac{1}{2} \log_e \sigma^2$	$\frac{1}{2} (1/n)$
Within months - -	1466	27.29	1.6533	.00035
Between months -	48	239.68	2.7397	.01042
Difference = $z = 1.0864$				Sum = .01077 $\sigma_z = .10376$

For the difference to be significant  $z$  should be about 1.65 times its standard error so that in this case the difference is very significant. In other words, Octobers really do differ significantly in their maximum temperatures.

Table VII. shows  $z$  and its standard error for the four measures of temperature under consideration for each month. It will be noticed that although the between-month variance is always significantly different from that within months,  $z$  is much greater in some months than in others.

For example, the maximum temperatures in one May tend to be much more like those in other Mays than those in January, which tend to vary greatly from year to year. Again, there is much more variation in the minimum temperatures from January to January than there is from June to June. The maximum temperature is always more variable from year to year than the minimum.

TABLE VII.—THE SIGNIFICANCE OF THE " BETWEEN-MONTH " VARIANCE  
ROTHAMSTED 1878-1926.

	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.
<i>Variance of Maximum Temp.</i>												
Within Months . . . .	28.32	27.20	27.81	33.43	33.03	30.07	36.45	36.67	45.79	36.85	20.83	26.30
Between Months . . . .	245.01	239.68	233.69	355.45	403.29	382.11	319.64	200.25	234.22	220.12	429.87	300.12
Z	1.079	1.086	1.064	1.182	1.251	1.271	1.086	0.980	0.816	0.894	1.334	1.217
<i>Variance of Minimum Temp.</i>												
Within Months . . . .	34.55	41.38	38.37	41.16	43.98	39.11	32.20	28.33	31.41	21.75	17.32	19.48
Between Months . . . .	77.34	247.14	205.16	350.45	341.22	319.20	172.30	101.67	112.86	63.40	81.04	73.33
Z	0.403	0.894	0.838	1.071	1.024	1.050	0.839	0.639	0.640	0.535	0.772	0.663
<i>Variance of Mean of Maximum and Minimum Temp.</i>												
Within Months . . . .	22.20	26.27	26.43	30.56	31.85	27.00	25.51	22.58	28.73	19.36	15.17	14.32
Between Months . . . .	108.85	216.16	208.97	333.14	346.79	327.88	202.89	116.82	137.50	105.21	196.17	137.42
Z	0.795	1.054	1.033	1.194	1.193	1.232	1.037	0.822	0.783	0.846	1.280	1.130
<i>Variance of Range</i>												
Within Months . . . .	36.92	32.25	26.63	26.96	26.64	26.77	35.24	39.71	39.47	39.76	33.63	34.28
Between Months . . . .	209.30	109.02	41.83	79.24	101.86	91.09	172.30	256.56	144.16	146.19	237.14	197.21
Z	0.867	0.609	0.226	0.539	0.671	0.612	0.794	0.933	0.648	0.651	0.977	0.875
<i>Degrees of Freedom</i>												
Within Months . . . .	1419	1466	1420	1468	1432	1306	1437	1391	1436	1392	1439	1439
Between Months . . . .	48	48	48	48	47	47	47	47	47	47	47	47
Standard Error of Z	0.1038	0.1037	0.1038	0.1037	0.1048	0.1050	0.1048	0.1049	0.1048	0.1049	0.1048	0.1048

The range is much less variable than the other measures. Indeed in one month, November, it is only just significant. It is, on the other hand, an interesting fact that the range should be significantly variable between months at all.

#### EXAMINATION OF RESULTS IN DETAIL.

Having examined the significance of the year-to-year variance the actual results may be examined in detail, the day-to-day variation being considered separately from that between months. Fig. 2 shows the actual mean monthly temperatures over the

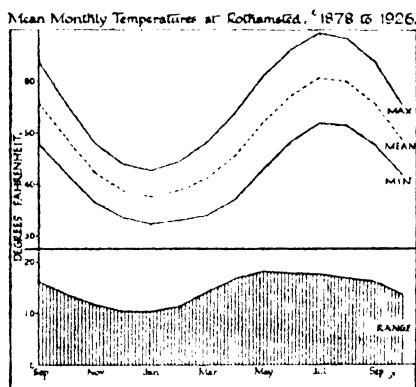


FIG. 2.

whole period. The maximum and minimum are shown and the mean of the two. Below is shown the range.

The most interesting part of this diagram is the last. It is curious to note that whereas in summer the range is high, as might be expected, it is comparatively small in the winter; the tendency is apparently for a fall in night temperature (represented by the minimum) in winter, to be accompanied by a similar fall in the day (represented by the maximum). In the summer (and particularly in May), on the other hand, a rise in the maximum temperature does not mean necessarily a similar rise at night. This is very fully borne out by the diagrams to be presented dealing with the variance and correlations between the two measures.

Table VIII. shows the variances and coefficients of correlation from day to day within months for the maximum and minimum temperatures, the mean of the two, and the range between the two.

To the logarithms of these values smooth curves have been fitted and these are shown in Figs. 3 to 6. The advantages of using the logarithms are twofold.

In the first place a common standard error can be found for each curve, which is independent of the variance itself and thus the significance of any difference can be seen at once. Also, differences between the lower values of the variance are exaggerated and between the higher values lessened, this giving a truer picture of the state of affairs.

TABLE VIII.—VARIANCE OF AND CORRELATIONS BETWEEN MAXIMUM AND MINIMUM TEMPERATURES FROM DAY TO DAY WITHIN MONTHS.

	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.
Total No. of Days . . . . .	1468	1515	1469	1517	1480	1354	1485	1439	1484	1440	1487	1487
Variance within months of :												
Daily Maximum Temperature . .	28.3	27.3	27.8	33.4	33.0	30.1	36.5	36.7	45.8	36.9	29.8	26.3
Daily Minimum Temperature . .	34.6	41.4	38.4	41.2	44.0	39.1	32.2	28.3	31.4	21.8	17.3	19.5
Daily Mean = $\frac{1}{2}$ (Max. + Min.) . .	22.2	26.3	26.4	30.6	31.9	27.9	25.5	22.6	28.7	19.4	15.2	14.3
Daily Range = (Max. - Min.) . .	36.9	32.3	26.6	27.0	26.6	26.8	35.2	39.7	39.5	38.8	33.6	34.3
Correlation between :												
Maximum and Minimum Temps. .	+415	+542	+605	+642	+661	+618	+488	+393	+498	+356	+297	+254
Mean and Range . . . . .	-109	-242	-199	-135	-188	-165	+071	+139	+213	+272	+277	+154

If  $z$  is taken equal to half of the difference of the common logarithms as before, then since in this case common logarithms are used, the standard error of  $z$  must be multiplied by .4343, or,

$\sigma_z = .4343 \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$ . In the cases dealt with a common  $\sigma_z$  for each curve has been worked out based on the mean number of degrees of freedom. The loss of accuracy is small.

In the case of the correlation coefficient  $r$ . In this case,

$$z = \frac{1}{2} \{ \log_e (1+r) - \log_e (1-r) \}$$

This serves to exaggerate the differences between higher values of  $r$  while leaving the lower values practically unchanged. Again a constant standard error wholly independent of  $r$  may be used:—

$$\sigma_z = \sqrt{\frac{1}{n-3}}$$

#### VARIANCE FROM DAY TO DAY WITHIN MONTHS.

Fig. 3 shows the variance of the maximum and minimum temperatures from day to day within the month.

In the case of the maximum temperatures the period of greatest variation is during the spring and early summer months. The most even months are August, September and October, after which there is a gradual rise in variance through the winter. The tendency here then is for the maximum temperature to vary very considerably from day to day in any one year in the spring and to be most even during the early autumn. The winter months are intermediate. The minimum temperature is exceptionally even during the summer months, and this influence will be seen to be of very considerable moment in the relationships to be discussed. October, November and December are all very variable in their minimum temperatures. After this there is a tendency for these temperatures to become more even again, broken only by a rather high variation in May, probably due to the possibility of late frosts occurring in the beginning of the month, together with warm nights towards the end. It may be noted here that a portion of this high variation, both in maximum and minimum temperature, during May is due to the natural rate of increase during the month; this factor, however, has really a comparatively small effect on the total variance for the month.

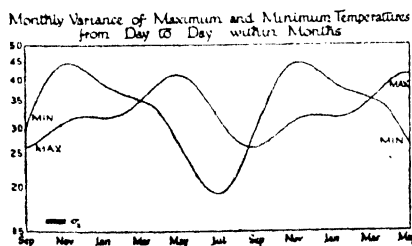


FIG. 3.

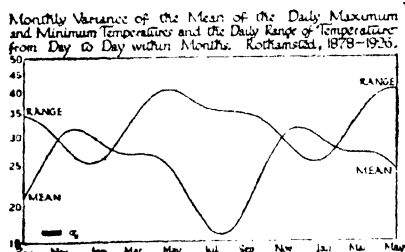


FIG. 4.

September behaves in a similar way to May in the minimum temperatures, but the effect is not felt in the maximum.

The variance of the mean of the two measures and that of the range from day to day is shown in Fig. 4. At first sight that for the mean apparently follows the curve for the minimum temperature very closely although it is always lower. Indeed, in the winter months where the maximum temperature is relatively constant, the minimum is the controlling factor in the variation of the mean and this is continued until May. In May both maximum and minimum are very variable and a very big rise in the variance, which is not brought out by the curve, results in the mean. After May both curves tend to fall, that for the maximum being rather less steep; here the mean is intermediate in slope, though of course less in magnitude than either. In the autumn while both rise, the minimum again seems to have the greater influence on the mean. This curve serves to show that, at any rate in winter, the mean is a somewhat unsatisfactory measure of the variation in daily temperature.

The range, it will be remembered, is highest in the late spring and summer and lowest in the winter. Its variance seems to follow this curve very closely and to have little relation to that of the maximum and minimum temperatures. The only real divergence is in September, when, probably, owing to the occasional occurrence of high day temperatures in the same twenty-four hours as cold nights, the variation is high.

#### CORRELATIONS FROM DAY TO DAY.

The correlation between maximum and minimum (Fig. 5) emphasizes some of the points already raised. It is, it will be noticed, always positive, but high in the winter and low in summer. May, with its exceptionally high variation, causes a secondary peak in the curve. In winter the tendency is for cold nights to be accompanied by cold days, but this tendency decreases in the spring. In the summer, however, the minimum is, as has been seen, exceptionally invariable and the tendency is for a hot summer day to have but little impression on the temperature at night. It may

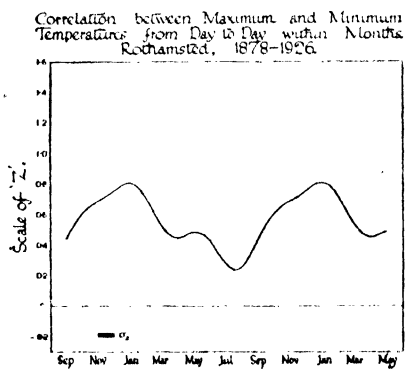


FIG. 5.

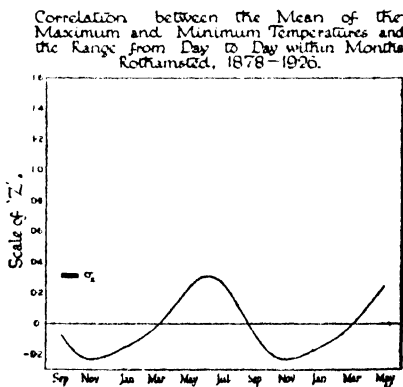


FIG. 6.

be observed that the occurrence of a minimum temperature over  $60^{\circ}\text{F.}$  is very rare at this station. During the whole forty-eight years there have been only 37 occasions when this has happened, while the maximum temperature has been over  $80^{\circ}\text{F.}$  163 times. On the other hand, during June, July and August forty per cent. of the minimum readings have been between  $49^{\circ}\text{F.}$  and  $53^{\circ}\text{F.}$

The curve (Fig. 6) showing the correlation between the daily mean and the daily range is amazingly regular.

In the winter the lower the mean temperature the wider is the range. In the summer, on the other hand, the correlation between the two is positive. This is closely connected with the fact that in the winter the minimum temperature is more variable than the maximum; that is to say, although a fall in the day temperature generally accompanies a big drop during the night, the day decrease is not nearly so great.

In the summer the position is reversed, since, as has been shown, a very hot day is not necessarily accompanied by a more than ordinarily warm night.

In the spring and autumn the correlation between the mean and the range is barely significant.

#### VARIAION OF MONTHS FROM YEAR TO YEAR.

The variance and correlations of the above measures from year to year are shown in Table IX.

To these also, curves have been fitted and are shown in Figs. 7 to 10. In considering these curves it must be remembered that, while the within-year variances were based on between fourteen and fifteen hundred days, those between years are only based on forty-seven or forty-eight years. Differences in order to be significant, have to be considerably larger in consequence than in the former case.

The variance curves for the maximum and minimum are of great interest (Fig. 7). It will be noticed that the maximum temperature is always very variable from year to year. Curiously enough, however, the spring and autumn months, which are most variable from day to day are distinctly less variable than the winter

Monthly Variance of Maximum and Minimum Temperatures from Year to Year. Rothamsted, 1878-1926.

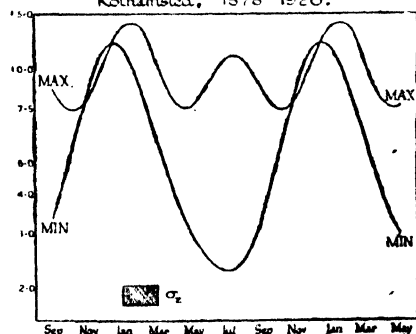


FIG. 7.

Monthly Variance of Mean of Maximum and Minimum Temperatures and the Range from Year to Year Rothamsted, 1878-1926.

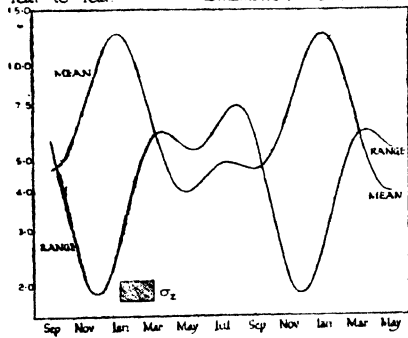


FIG. 8.

TABLE IX.—MONTHLY VARIANCES OF AND CORRELATIONS BETWEEN MAXIMUM AND MINIMUM TEMPERATURES FROM YEAR TO YEAR.

	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May	June	July	Aug.
Total number of years . . .	49	49	49	49	48	48	48	48	48	48	48	48
Variance "between Months" of:												
Maximum Temperature . .	8.17	7.73	7.79	11.46	13.01	13.65	10.31	8.67	7.55	7.34	13.87	9.68
Minimum Temperature . .	2.58	7.97	6.84	11.30	11.01	11.40	5.56	3.39	3.64	2.11	2.61	2.36
Mean = $\frac{1}{2}$ (Max. + Min.) . .	3.63	6.97	6.97	10.75	11.19	11.71	6.55	3.89	4.44	3.51	6.33	4.43
Range = (Max. - Min.) . .	6.98	3.52	1.39	2.55	3.29	3.25	5.56	8.55	4.65	4.87	7.65	6.36
Correlation between :												
Maximum and Minimum Temperatures . .	+ .411	+ .776	+ .907	+ .888	+ .866	+ .874	+ .681	+ .324	+ .624	+ .581	+ .733	+ .594
Mean and Range . . .	+ .555	- .024	+ .153	+ .015	+ .165	+ .182	+ .394	+ .458	+ .431	+ .632	+ .809	+ .689

and summer months. If May and July are compared it will be seen that, whereas May if compared with July is an exceedingly variable month from day to day, the mean maximum temperature is much less variable from year to year. July, on the other hand, seems to be much more even than May from day to day, but considerably more variable from year to year.

Again, December tends to be a warm month or a cold month, but not to vary much within the month.

We have seen that the minimum temperature varies little from day to day in the summer. This is also apparent from year to year. It does not seem to matter what happens to the day temperature, not only in any one summer, but every summer, the night temperature is not appreciably affected.

In the winter, on the other hand, there is not only a high variation from day to day in the minimum, but also from year to year.

Since maximum and minimum temperatures are highly correlated in winter and are both very variable, the mean (Fig. 8), naturally, is also very variable from year to year, giving probably a very fair estimate of the temperature at this time.

In the summer, however, the difference between a hot and a cold season is only clearly shown by the day temperature; thus it is the variance of the maximum that most influences the mean at this time, being in fact low through spring, summer and autumn with a maximum in July. The whole variance of the mean, however, is steadied down by the even night temperatures.

The range curve is nearly the converse of that for the mean. In winter a low maximum is accompanied by a low minimum, but in the summer, however, the range is much more variable from year to year.

#### CORRELATION FROM YEAR TO YEAR.

From year to year the maximum and minimum temperatures (Fig. 9) are very highly correlated in the winter months; much more so than from day to day. Thus a cold December is reflected both in the minimum and maximum temperatures.

The correlation in the spring and autumn is still positive but low. Thus, while in April a series of warm days may increase the

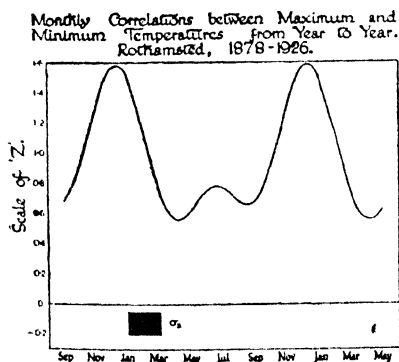


FIG. 9.

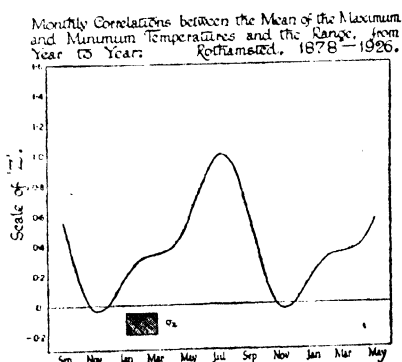


FIG. 10.

mean maximum temperature, it does not necessarily follow that spring frosts will not occur at night and lower the average for the minimum.

There is a distinct rise in the summer indicating that, while a hot summer day is not necessarily accompanied by a warm night, in a hot July, the mean minimum temperature is raised, though as has been seen, not on anything like the same scale as the maximum.

Owing to the high correlation between the maximum and minimum temperatures from year to year the tendency for mean and range (Fig. 10) to be negatively correlated in winter from day to day is somewhat swamped. In consequence during the winter months there is no significant correlation between the two from year to year. In the summer and early autumn, however, the correlation between maximum and minimum is low from year to year and thus the correlation between mean and range is high and positive.

Thus the mean temperature of a winter month does not appreciably affect the range, while in the summer hot months are certainly accompanied by large differences between maximum and minimum temperatures.

#### SUMMARY.

1. It is shown that owing to the bulk of data accumulated, it was necessary to condense the daily readings of maximum and minimum temperature before an adequate statistical analysis could be attempted. The daily readings were therefore reduced to five constants for each month during the forty-eight years under consideration. These were the mean and variance for maximum and minimum temperatures and the product moment for the two sets of readings.

2. The method by which this was carried out is given in some detail in the hope that other stations will also carry out this summarization and analyze their data in a similar way.

3. The data are analyzed, use being made of the method of *Analysis of Variance* developed by R. A. Fisher.

4. It is demonstrated that the variation over a period of years is divided into two portions, that within months and that between the same months in different years. The significance of this "between-month" variation is examined, and it is shown that all months vary significantly from year to year, but that in certain months this variation is much greater than in others.

5. The results of the analysis are presented in the form of a number of diagrams. These show, firstly, the normal variation of the maximum and minimum temperatures from day to day within each month. It is seen that the greatest variation of the maximum temperature is found in the spring and early summer, the winter being comparatively even; the minimum temperature on the other hand varies greatly in the winter months, while the summer months are very even. Secondly, the variation of the daily mean and the range "within months" is discussed. It is apparent that the variation of the mean temperature is greatly influenced by the low variation of the minimum during the summer months. The mean tends, therefore, to give a poor idea of fluctuations of temperature

during this time. Thirdly, diagrams are given illustrating the correlations from day to day between the maximum and minimum temperatures and also between the mean and the range. In the first case the correlation is always positive, but much higher in the winter months than in the summer; in the second, the mean is negatively correlated with the range in the winter and positively in the summer. The correlation is, however, always low.

Fourthly, diagrams are given illustrating the variation in maximum and minimum temperatures, mean and range from year to year. It is pointed out that whereas in the spring and autumn months the variation of the maximum temperature within months is high, the mean maximum temperature varies comparatively little from year to year. In the winter and summer, however, the maximum varies very considerably between years.

The minimum temperature has its greatest variation from year to year in the winter, but has an extraordinarily low variance in the summer months.

It is shown that the mean of the maximum and minimum probably gives a fair idea of the variations in temperature from year to year in the winter months, but that the maximum temperature most influences the mean during the summer months.

The variation of the range is apparently very nearly the converse of that for the mean.

Finally, the correlations from year to year between maximum and minimum and also mean and range are discussed.

In conclusion, the writer would like to acknowledge his indebtedness to the Ministry of Agriculture, who have enabled him to spend the last year at Rothamsted; also he would like to thank Dr. Fisher, who inspired this work and whose sympathy and advice have always been readily given.

## DISCUSSION.

SIR RICHARD GREGORY: I am sure you will all agree with me that Mr. Hoblyn's paper is of a type to be welcomed at our meetings and for publication in our *Journal*. It is of interest to meteorological observers and of value also to horticulturists and farmers. Every crop has its own range of temperature for growth and development, and to know in any particular district the probable and extreme variations month by month, as illustrated by Mr. Hoblyn's diagrams, would be of the greatest practical service. It is evident from the tables and the diagrams that May deserves its reputation for fickleness of temperature. Rothamsted, where the observations analysed were made, has a heavy soil, and the response of plants to temperature changes would probably be different there from what it would be in a soil of greensand or chalk; nevertheless, it would be a decided advantage to know the prevalent weather in any district month by month, and also the range of the possible variations. The material for making such an analysis of temperatures in the chief districts is in the possession of the Meteorological Office, and if the work could be done it would make a very valuable supplement to the *Book of Normals*. It would be necessary, however, to present the facts in a way intelligible to the lay mind and without the use of technical terms or formulæ. What are wanted in this and other scientific subjects are transformers who can reduce these high tension currents to voltages

suitable for everyday needs and uses. There are probably several Fellows present who will be able to make useful observations upon some of the points in Mr. Hoblyn's interesting paper and to join with me in expressing our thanks for his account of it.

Mr. W. H. PICK said that he wanted to congratulate the author very heartily upon two counts: first, for the enormous amount of work that the paper manifested; and secondly, for the large quantity of really useful information that was summarised into such a readily perceivable and digestible form. He felt, too, that it was very good to see this new marriage of meteorology with agriculture, because the dependence of the latter upon the former was so great. Years ago they had been very closely linked, but something in the nature of a divorce had taken place in the interval. Papers like the one now being discussed were very valuable for the agriculturist and it was to be hoped that more with an agricultural bias would be forthcoming as a large proportion of the Society's Fellows had agricultural connections; that would mean that the interest of these Fellows would be heightened as well as fruitful work being done.

Mr. L. C. W. BONACINA remarked on the overwhelming complexity of agricultural meteorology. There was no doubt that a Rothamsted was needed in every English county. The optimum conditions of rainfall and temperature for different crops varied from place to place within a few miles in accordance with changes of soil and other factors, and one had to bear in mind, as Sir Napier Shaw had remarked, in any particular season the influence of the previous season, and so forth.

Some of the statistical results brought out for Rothamsted were reflected in general experience with surprising clearness. Take, for instance, the contrast drawn between May and July. It was very apparent that May, although a month of violent vicissitude of heat and cold from day to day, was quite a constant month, so far as any month could be so-called in England, from year to year. On the other hand, July, though comparatively steady from one day to another, was a most uncertain and eccentric month from one year to another, one July being relentlessly hot and dry, another hopelessly wet and chilly.

Other Fellows who took part in the discussion were Dr. G. C. SIMPSON, Dr. C. E. P. BROOKS, and Sir NAPIER SHAW.

## ON THE ANNUAL REVISION OF FORECASTING FORMULAS BASED ON PARTIAL REGRESSION EQUATIONS

By A. J. PAGE, *Rothamsted Experimental Station, Harpenden, Herts*

The fitting of regression lines to statistical data which have been obtained by experiment or other means more or less under control, has been widely applied, and has produced valuable results, but the method is much more restricted when applied to statistics relating to economic and social problems on account of the complexity and variety of the underlying causes at work. Cases, however, do arise where the main factors are few and easily distinguishable, thereby enabling exact mathematical procedure to be followed with some degree of success. It has been found possible, for example, to work out prediction formulas for the preparation of official crop forecasts, though a difficulty often arises owing to the comparative shortness of the time series available as a basis of the calculations, which may be due not to the absence of figures for earlier years, but to changes in conditions during the course of time which would render the material of a long series obviously heterogeneous. When all such causes and conditions have reached a fairly stable state of equilibrium amongst themselves, the addition of each successive year's data, as time goes on, lengthens the series available, and enables the dependent variate to be determined with greater precision.

The procedure to be followed is simple and does not involve the use of correlation coefficients. It may be studied in full in such a work as R. A. Fisher's *Statistical Methods for Research Workers*. The arithmetical work involved may, however, be laborious, and the object of the present note is to show how, when once the necessary equations have been obtained from the data available for any given period of years, these equations may easily be brought up to date by the incorporation of the figures for a new year, without having to recalculate *ab initio* all the numerical coefficients involved.

If the expected value of a dependent variate  $Y$  is expressed in terms of a number of independent variates  $x_1, x_2, x_3, \dots, x_r$  in the form of a partial regression equation

$$Y = b_1x_1 + b_2x_2 + \dots + b_r x_r, \quad (1)$$

the partial regression coefficients  $b_1, b_2, \dots, b_r$  are obtained from the simultaneous equations

$$\left. \begin{array}{l} b_1\Sigma(x^2_1) + b_2\Sigma(x_1 x_2) + . . . . + b_r\Sigma(x_1 x_r) = \Sigma(x_1 y) \\ b_1\Sigma(x_1 x_2) + b_2\Sigma(x^2_2) + . . . . + b_r\Sigma(x_2 x_r) = \Sigma(x_2 y) \\ . . . . . \\ b_1\Sigma(x_1 x_r) + b_2\Sigma(x_2 x_r) + . . . . + b_r\Sigma(x^2_r) = \Sigma(x_r y) \end{array} \right\} \quad (2)$$

The system of equations (2) may then be brought up to date merely by calculating for each variate the difference between  $t$  times its new value and the sum of the previous values, and adjusting the coefficients by the addition of the terms given by (3) and (4).

As a practical example of the method, figures used for the official forecasts of the exportable surplus of rice from the province of Burma may be cited. The full set of equations for the whole province are too elaborate to be quoted here, as no fewer than six  $b$ 's in six simultaneous equations are involved, but the subsidiary equations from the port of Akyab which deal exclusively with the produce of the rice lands in the Arakan division of the same province may be quoted as they involve only two parameters. For the years 1902 to 1926, omitting the years 1915 to 1920 which were affected by war conditions, the exportable surplus is given by the equation

$$Y = -27.390 + .257x + .029x'$$

where  $Y$  is the difference in hundreds of tons between the exports of any year and those of the preceding year,  $x$  is the difference between the area harvested in hundreds of acres multiplied by the condition figure of the crop for the same year, and the corresponding product of the preceding year, whilst  $x'$  is the similar difference between the similar products for the preceding and second preceding year. In other words, and expressed algebraically, if  $a_1, a_2, a_3$  are the areas harvested in three consecutive years and  $c_1, c_2, c_3$  the corresponding condition figures of the crops

$$x = a_3 c_3 - a_2 c_2, \quad x' = a_2 c_2 - a_1 c_1.$$

Now the coefficients .257 and .029 were obtained as the approximate solutions of the equations

$$\begin{aligned} 19,295,470b_1 - 11,971,249b_2 &= 4,608,535 \\ -11,971,249b_1 + 33,387,878b_2 &= -2,096,725 \end{aligned}$$

where the coefficients are the sums of squares, or products, for 17 years.

The figures for the succeeding year 1926-27 were  $x = -219$ ,  $x' = -354$  and  $y = -280$ ;  $t$  is 17,  $t+1$  is 18 and the crude totals  $\Sigma(x)$ ,  $\Sigma(x')$  and  $\Sigma(y)$  for 17 years were 6,368, 1,702, and 1,285 respectively. For the three variates  $x$ ,  $x'$  and  $y$  we have therefore

$$\begin{aligned} 17 \times (-219) - 6,368 &= -10,091 \\ 17 \times (-354) - 1,702 &= -7,720 \\ 17 \times (-280) - 1,285 &= -6,045 \end{aligned}$$

of which the squares and products divided by  $17 \times 18 (= 306)$  gives the adjustments to the coefficients:

$$\begin{array}{r r r} 332,772 & 254,583 & 199,347 \\ 254,583 & 194,766 & 152,508 \end{array}$$

and on adding these to the coefficients of the old equations we arrive at the following new equations

$$\begin{aligned} 19,628,242b_1 - 11,716,666b_2 &= 4,807,882 \\ -11,716,666b_1 + 33,582,644b_2 &= -1,944,217, \end{aligned}$$

giving a new regression equation

$$Y = -37.551 + .266x + .035x'.$$

The benefit of this simple labor-saving formula is not, however, seen in its fullest extent in the example given since considerations of space prevented heavier work being displayed.

A further advantage of the method is that in some cases, as in the one given above, where the  $x$ 's are known early in the year but the  $y$ 's are not known until the year has closed, the equations can be revised, as far as all the coefficients on the left-hand sides of the equations are concerned, and kept in readiness for the insertion at the earliest possible moment of the corrections to the  $\Sigma(xy)$  items, when the new solutions for the  $b$  parameters can quickly be found. Indeed, using the left-hand sides of the equations only, a matrix of multipliers may be prepared, which, with the new values for the right-hand sides, will supply the new solutions by direct multiplication and addition, thus anticipating nearly all the labor, not only of preparing, but of solving the equations.

# REVISED OFFICIAL METHOD FOR THE MECHANICAL ANALYSIS OF SOILS

*Adopted by the Agricultural Education Association in 1927*

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## INTRODUCTION

The Agricultural Education Association adopted in 1925 a new official method of Mechanical Analysis to replace the older sedimentation method adopted in 1906. The essentials of the new method consisted in (a) the use of hydrogen peroxide to destroy organic matter and thus to aid the complete dispersion of the soil, and (b) a shorter procedure to obtain the percentage of the fractions, depending on the measurement by pipette sampling of the depth-concentration relationship in a settling suspension. Both these improvements, which were introduced by Robinson (*J. Agric. Sci.*, Vol. 12 (1922), pp. 287 and 306), were thoroughly examined by a sub-committee of the Association, consisting of Professor N. M. Comber, Dr. B. Dyer, Professor J. Hendrick, Professor G. W. Robinson, Mr. T. Wallace, with Dr. B. A. Keen as convener. The extensive experimental work was carried out at Bangor, Leeds and Rothamsted, under the direction of Professor Robinson, Professor Comber and Dr. Keen respectively. A full account of this work and a detailed discussion of the position of mechanical analysis at that time appeared in the *Journal of Agricultural Science*, Vol. 16 (1926), p. 123. The full working details of the method were published in *Agricultural Progress*, Vol. III, 1926.

Since this date, Commission 1 of the International Society of Soil Science has met at Rothamsted (October, 1926), where it discussed the question of issuing an agreed international method of mechanical analysis. Certain conclusions were reached, which were adopted with only minor modifications by the full Congress of the International Society of Soil Science at Washington, D.C., in June, 1927.

The full details of the International method will be found in the Proceedings of the Congress. It agrees with the British method in the preliminary treatment and dispersion of the soil and in the use of the pipette method of sampling, but differs from it in that (1) the percentages of fractions are expressed on the oven-dried (105° C.) and not on the ignited basis, and (2) the settling times used for the separation of the fractions are different.

The Chemistry Committee of the Agricultural Education Association at its meeting in December, 1927, discussed these differences, and instructed the Mechanical Analysis Sub-committee to revise the British method so as to bring it into line with the International method, unless, on further consideration, there appeared to be insuperable objections to this course. The Sub-committee accordingly recommends that the method, given in detail below, shall become the Revised Official Method of the Association. In making this recommendation the Sub-committee has given due weight to the fact that no large number of analyses has yet been accumulated by the official method adopted in 1925.

The changes necessitated, together with the reasons for them, are set out below.

### 1. *The Expression of the Fractions as Oven-dried and not Ignited.*

The British practice of igniting the fractions before weighing stands almost alone. It was introduced with the 1906 method because of the incomplete removal of humus in the pre-treatment. The introduction of the peroxide method for the express purpose of removing the bulk of the organic matter has made the subsequent ignition very largely unnecessary, but it was retained in the 1925 official method owing to the large number of analyses already accumulated on an ignited basis. It now appears that the question of the ignition of a soil has fallen into a different perspective. Its use lies no longer in giving a truer series of values for the percentages of the fractions present in a soil; but it has become rather a means of ascertaining (after allowance for the humus content as given by the peroxide treatment) some information about the water of constitution of the clay and allied properties. As such the loss on ignition figure is desirable, and, although it may well be continued as a standard routine determination, it should in future be recorded separately from the actual mechanical analysis figures.

The employment of oven-drying instead of ignition will considerably increase the figures for the percentage of soil fractions, and this increase will fall to the

greatest extent on the clay. Those workers who wish for any purpose to secure ignited fraction weights for their own use can, after the oven-dry weights have been obtained, carry the same fractions through to ignition. Alternatively, a determination of the loss on ignition can be made on a separate portion of the soil and this figure set off against the percentage of oven-dry clay, on which, as stated, the ignition loss predominantly falls.

## 2. The Diameters and the Number of the Fractions

The present, and proposed settling depths and times, and the assumed maximum diameter of each fraction are as follows:—

PRESENT SCALE			
	Depth.	Time.	Corresponding Diameter.
Clay . . .	8.6 cms.	24 hrs.	0.002 m.m.
Fine silt . .	12.0 "	20 mins.	0.01 "
Silt . . .	30.0 "	5 "	0.04 "
Fine sand } Coarse sand }	Separated by the 0.2 m.m. sieve		{ 0.2 " 1.0 "
INTERNATIONAL SCALE			
Clay . . .	10 cms.	8 hrs.	0.002 m.m.
Silt . . .	10 "	4 mins. 48 secs.	0.02 "
Fine sand } Coarse sand }	Separated by the 0.2 m.m. sieve		{ 0.2 " 2.0 "

The figures refer to a temperature of 20° C. for the International scale, and 15° C. for the present scale.

In each case, these diameters are conventional figures and bear only a rough relation to the actual sizes of the particles. They are calculated, of course, from Stoke's Law, and are only of use as a kind of mental picture of the grain sizes. It is the settling velocity rather than the diameter that is important. In the case of the new method, a particle that settles 10 cms. in eight hours (in water at 20° C.) is defined as 0.002 m.m. equivalent diameter, and the diameters of all other fractions are calculated from Stoke's Law on this basis. In the 1925 official method, the conventional diameters and the settling velocities are not so logically related, as can easily be seen by a few test calculations. The diameters were, in fact, altered empirically from microscopic observation of the particles at the time the 1906 method was introduced, and these conventional diameters were retained in the official method adopted in 1925. Thus for the clay the same conventional diameter is employed as in the proposed new method, although the settling heights and times (at 20° C.) are respectively 9.8 cms. in 24 hours (or 8.6 cms. at 15° C.) in the present method, as against 10 cms. in 8 hours in the new method. The clay fraction in the new method, in fact, includes some of the finer portions of the old "fine silt" fraction. From both physical and chemical reasons there is justification for this inclusion, and many institutions make a practice of dividing the fine-silt fraction into two, so that the amount of the finer portion of the fine-silt fraction can, when desired, be added on to the clay. In any case, the change in the clay figure occasioned by the proposed alteration in settling velocity is small, amounting only to a few per cent on a very heavy soil, while for the majority of soils the difference is negligible. A further advantage in the shorter settling time is that those employing the beaker sedimentation method can obtain two pourings in 24 hours instead of one only as at present.

The other fractions call for little comment. There is one less in the new method, thus reducing the labour of routine work, and for special purposes the experimenter can always introduce additional settling times and subdivide the fractions to any desired degree. The 1 m.m. sieve has been replaced by a 2 m.m. sieve for defining the upper limit of the soil—as distinct from stones and gravel that are determined on a separate bulk sample—in view of the very general use of this size in other countries.

A further very strong argument for the new scale of fractions is that they are widely employed in our Dominions and Colonies. There can be no question of the desirability of a uniform scale for mechanical analysis in the Empire, and

the advantage of securing this and, at the same time, conformity with the International method is very great indeed. Finally, the method of summation curves can be employed if it be desired to transfer an analysis by the new method to the older British scale. (See Note G.)

### 3. *The Determination of Air-dry Moisture Content in the Soil Sample*

The International method prescribes that all figures shall be expressed as a percentage of the dry soil. Unless the soil is so dried before sampling, which is liable to introduce difficulties in the dispersion, it is necessary to work on air-dried soil and to recalculate the results to an oven-dry ( $105^{\circ}\text{C.}$ ) basis. The determination of the air-dry moisture content is a valuable feature of the British method, and this determination should be retained and included in the figures. There is the more reason for this, in that results so reported can be transferred by the reader to the oven-dry basis by a simple calculation.

#### DETAILS OF METHOD

On arrival at the laboratory the soil is spread out to dry at room temperature, and is then passed through a 2 m.m. round-hole sieve, any lumps being previously gently disintegrated in a mortar with a wooden pestle. The residue on the sieve, consisting of stones and fine gravel, before being discarded, is weighed and expressed as a percentage of the total weight of the air-dry soil. The material passing the sieve is the bulk sample from which separate sub-samples are carefully and uniformly taken for the following analyses:—

- I. Grading into fractions.
- II. Determination of carbonates.
- III. Moisture content, and, if desired, loss on ignition.

The working details for each of the three above groups are given below.

#### I. *Grading into Fractions*

This section consists of two distinct operations, (a) dispersion, and (b) the actual mechanical analysis.

(a) *Method of dispersion.* Weigh out 20 grams of air-dry soil into a tall 600 c.c. beaker. Add about 60 c.c. of 6 per cent (20 vol.) hydrogen peroxide and place on a hot plate, or over a rose burner. A vigorous reaction with organic matter soon takes place. The contents are stirred from time to time, and watched to avoid frothing over. After the reaction has subsided a further addition of peroxide should be made and the beaker again heated. There is usually no great degree of frothing with the second addition of peroxide, unless the soil contains much organic matter, when further additions of peroxide must be made. The humified organic matter is rendered soluble or destroyed by the peroxide treatment, and the soil loses its dark humic colour. After cooling, enough water and HCl is added to give 150–200 c.c. of N/5 HCl, allowance being made for the acid necessary to decompose carbonates. The contents are allowed to stand, with frequent stirring, for about an hour (or left overnight) and then filtered through an 18-cm. hard filter paper. If Whatman No. 1 be used, the first runnings may be turbid, but by pouring them back on to the filter, a clear filtrate may be obtained. The filtration is expedited by using a filter pump and hardened paper. The soil is then thrice washed on the filter with 100 c.c. of distilled water each time. No attempt should be made to wash down the soil to the bottom of the filter, as not only does this considerably decrease the rate of filtering, but fine material may pass through. If this washing has not made the soil practically free from electrolytes, it must be continued. After washing is complete, the filter paper is spread out on a large watch glass, and the soil washed with a jet of hot water on to a wire-mesh sieve with square apertures of 0.2 m.m. wide held over the mouth of a beaker. When no more can be removed, the paper is rolled into a loose ball, thoroughly wetted, and squeezed like a sponge. This process is repeated as long as any turbid liquid can be obtained. The material that cannot be removed in this way does not exceed 0.2–0.4 per cent of the whole sample.

The filtrate collected after the peroxide-HCl treatment contains, in addition to calcium from calcium carbonate, a small quantity of dissolved material, amounting usually to about 2–3 per cent of the total weight of soil, and consisting principally of mixed sesquioxides and a small amount of silica. For routine

purposes this loss of sesquioxides and silica may be determined by weighing, after gentle ignition, the precipitate obtained by the addition of ammonium hydroxide and ammonium chloride to the filtrate. It should be reported as "loss by solution." (See Note D.)

(b) *Mechanical analysis of dispersed sample by pipette method.* The material on the sieve is gently rubbed with a rubber pestle or the finger under a jet of water until no more will pass through. The residue on the sieve is the "coarse sand" fraction which is dried and weighed.

The material passing the sieve is transferred to a litre shaking bottle, and is made up to about 500 c.c. with distilled water, to which is then added 50 c.c. of dilute (10 per cent) ammonia, made by diluting 0.88 ammonia with twice its volume of distilled water. The contents are shaken in an end-over-end shaker at a speed of 30-40 revolutions per minute. For sandy soils overnight shaking is sufficient; very heavy soils may require forty hours. The shaking concluded, the suspension is made up to 1 litre in a measuring cylinder. The contents are thoroughly shaken by repeated inversion of the cylinder for about one minute, making certain that any sediment which has settled to the bottom is thoroughly mixed up with the liquid. The cylinder of suspension (equivalent to a 2 per cent suspension of the original soil) is then allowed to stand. The sampling can be done either with the special pipette and apparatus (see Note H), or alternatively a 20 c.c. pipette is fixed in a cork so that when the cork rests on the top of the cylinder, the point of the pipette will be the required distance below the original surface of the suspension.

The first sampling is made at the 10 cm. depth when the well-shaken suspension has stood for 4 minutes 48 seconds. The pipette, with the top closed, is lowered vertically into the suspension to the required depth (this operation is begun about 20 seconds before the time is up) and 20 c.c. of the suspension is withdrawn, care being taken to avoid too rapid ingress of the liquid, with consequent eddying in the bulk suspension.

The contents of the pipette are, if necessary, rapidly adjusted to the mark and delivered into a suitable tared dish. We have used and recommend the ordinary type of dish, 72 mm. diameter, commonly employed for the determination of dry matter in milk. The dish is then taken to dryness on a water bath, dried at 105° C. (see Note H), and, after cooling in a desiccator, weighed. The weight of material multiplied by five gives the concentration of the suspension at the point sampled. Let this be  $X$ . Then, the original concentration being 2 per cent, the percentage of material having settling velocity less than  $10/288 = 0.0347$  cm./sec. is given by  $100 X/2$ . Actually, the calculation given here in full reduces in practice to dividing the number of milligrams of the oven-dry material by four. This represents as a percentage the sum of the clay and silt.

The contents of the cylinder are again shaken up for one minute and allowed to settle for eight hours and sampled at a depth of 10 cm. below the new surface level, from which the percentage of clay is obtained, after drying, cooling and weighing as above. We have thus obtained the percentage of

silt + clay,  
clay,

and from these the percentage of silt is obtained by subtraction.

The remaining fraction, the fine sand, is determined directly. After the clay sampling, the bulk of the supernatant liquid is poured away and the sediment in the cylinder is transferred to a 400 c.c. beaker and made up with water to a height of 10 cms. above the base. It is well stirred up and allowed to settle for 4 mins. 48 secs., when the turbid suspension is poured away. The beaker is again filled to the mark with water and the process repeated until the liquid is no longer turbid at the end of the period. The residue is the fine sand, which is collected, dried and weighed as above.

(c) *Mechanical analysis by sedimentation method.* If no shaking apparatus is available, it may be necessary to carry out the mechanical analysis by the sedimentation method. In this case the material passing the 0.2 m.m. sieve is stirred up with 10 c.c. of 10 per cent ammonia and made up with distilled water to the 10 cm. mark. It is then poured off after 8 hours, and the pourings repeated until all clay has been removed. It is possible to make two pourings

in 24 hours, the beaker being filled up after each 8-hour pouring and allowed to stand overnight. It may not be practicable to fill the beaker to the full height corresponding to the night period, which will be about 16 hours; but if, say, 14 cms. is used instead of the necessary 20 cms., a considerable amount of clay will be removed and the time taken correspondingly shortened. The clay is determined directly, by evaporating an aliquot part to dryness at 105° C. and weighing. The residue in the beaker is again made up to 10 cms. with distilled water, and poured off after an interval of 4 mins. 48 secs. The process is repeated until all silt has been removed. This is collected, dried at 105° C. and weighed, and the final residue in the beaker, consisting of the fine sand fraction, is similarly dried and weighed.

It is to be observed that all the operations in (a), (b), and (c), can, if desired, be carried out on half the quantity of soil, i.e., 10 grms. In this case all other quantities are also halved, and a 500 c.c. cylinder is used in the pipette method.

## II. Determination of Carbonates

This determination may be made in the Collins calcimeter. It is unnecessary to give details here. Reference may be made to the description in *Journal Soc. Chemical Industry*, Vol. 25 (1906), p. 518.

## III. Moisture Content in Air-dried Sample

Weigh 10 grams of the air-dried soil into a wide weighing bottle and heat for 24 hours at 105° C., cool in a desiccator and reweigh. As a precaution, heavy soils and those containing appreciable amount of organic matter should be replaced in the oven for a further period of 24 hours and a second weighing made.

The loss on ignition of the soil, if required, can be determined on this oven-dry sample. (See Note C.)

## STATEMENT OF RESULTS

The results to be reported as percentages on the air-dry soil in the British Revised Official Method are therefore:—

- (1) Coarse sand, remaining on 0.2 m.m. sieve.
- (2) Fine sand, obtained by sedimentation.
- (3) Silt } obtained by pipette sampling.
- (4) Clay }
- (5) Moisture in air-dry soil.
- (6) Carbonates.
- (7) Loss by solution in peroxide-HCl treatment.
- (8) Difference (organic matter removed by hydrogen peroxide, and errors of experiment).

Total = 100.

Item (8) is the difference between 100 and the sum of the other seven items. The errors in working with oven-dry material will be mainly positive and may occasionally, in soils of low organic content, outweigh the organic matter dissolved by peroxide and acid. In this case the total of the first seven items will exceed 100, in contrast to the usual experience with ignited material, where the errors are mainly negative and lead to a total of less than 100.

Where it is desired to give results on an ignited as well as the oven-dry basis, the oven-dry fractions are taken to ignition after weighing, as already mentioned. The further results to be reported, calculated as percentage of the original air-dry soil, are then:—

- (1) Coarse sand, remaining on 0.2 m.m. sieve.
- (2) Fine sand obtained by sedimentation.
- (3) Silt } obtained by pipette sampling.
- (4) Clay }
- (5) Moisture in air-dry soil.
- (6) Carbonates.
- (7) Loss by solution in peroxide and HCl treatment.
- (8) Loss on ignition (organic matter and "water of constitution" of clay).
- (9) Difference (errors of experiment).

Total = 100.

A specimen result for the same soil (Pennant Grit, Glamorgan) on both the official oven-dry basis and the ignited basis is given below:—

<i>Revised Official Method.</i>		<i>Ignited Basis.</i>	
Coarse sand . . .	22.3	Coarse sand . . .	21.7
Fine sand . . .	32.7	Fine sand . . .	32.3
Silt . . .	18.2	Silt . . .	18.7
Clay . . .	16.8	Clay . . .	14.0
Air-dry moisture content .	3.1	Air-dry moisture content .	3.1
Carbonates . . .	0.7	Carbonates . . .	0.7
Loss by solution . . .	1.8	Loss by solution . . .	1.8
Difference . . .	4.4	Loss on ignition . . .	8.6
		Difference . . .	-0.9
Total . . .	100	Total . . .	100

#### NOTES

(A.) *Limitations of mechanical analysis.* Although the method described above is suitable for the great majority of soils, there are certain soil types for which any system based on particle sizes is not completely satisfactory. Soils very rich in organic matter or in calcium carbonate require special consideration. Moreover, in the case of highly calcareous soils there is difficulty in effecting dispersion. This can be met if after the peroxide treatment and the normal treatment with HCl the soil is then leached with HCl to remove absorbed or replaceable calcium. Another soil type that probably requires special consideration is that composed of unweathered and partially weathered rock particles. These, although relatively easily disintegrated, are not necessarily equivalent to the "compound particles" formed in the soil by cultivation processes that are completely dispersed before the analysis. Lateritic soils also need special attention. These are normally highly granulated, and methods sufficiently drastic to secure the prime particle structure would possibly attack unweathered material.

(B.) *The use of the "air-dry" moisture content.* If the usual "air-dry" moisture content be replaced by the loss in weight when the soil, previously brought to equilibrium over sulphuric acid of 50 per cent relative humidity, is transferred and brought to equilibrium over concentrated  $\text{H}_2\text{SO}_4$ , a definite value is obtained that has much to recommend it as a means of characterizing the soil (see *J. Agric. Sci.*, Vol. 15 (1925), pp. 68 and 272). Further, the reliability of the loss on ignition figure is improved by the greater definition of drying over concentrated sulphuric acid, as compared with oven drying. Sulphuric acid of 50 per cent relative humidity may be obtained by adding 23.4 c.c. of concentrated  $\text{H}_2\text{SO}_4$  to 57 c.c. of water and then adjusting, if necessary, to a density of 1.3325 at 15° C.

(C.) *Determination of loss on ignition.* A known weight of oven-dry soil is transferred to a small porcelain or vitrosil basin of known weight, ignited for thirty minutes in a muffle at a bright red heat (1000° C.), cooled and reweighed. The value obtained for the loss on ignition includes  $\text{CO}_2$  from the carbonates, and is therefore too high. When the carbonate content of the soil has been determined, a weight equal to 44 per cent of the carbonate should therefore be subtracted from the ignition value in order to obtain the true loss on ignition. If the ignition is carried out over a Bunsen at such a temperature that complete decomposition of  $\text{CaCO}_3$  into  $\text{CaO}$  and  $\text{CO}_2$  does not occur, it is advisable, after ignition, to moisten the residue with ammonium carbonate solution, and ignite gently to drive off excess of ammonium carbonate. After this treatment, the residue contains the original amount of calcium carbonate, and no correction of the loss on ignition value is therefore needed.

(D.) *Determination of silica and sesquioxides.* When the amounts of silica and sesquioxides in the filtrate after peroxide-HCl treatment are desired separately, the weighed precipitate, after  $\text{NH}_4\text{Cl}$  and  $\text{NH}_4\text{OH}$  treatment and ignition, as described in I (a), is treated with strong HCl, evaporated to dryness, and heated to 150° C. on a sand- or air-bath for two hours, to render the silica insoluble. It is then moistened with strong HCl and allowed to stand for ten

minutes. Water is then added, the liquid is cooled and the silica separated by filtration, well washed with hot water, dried, ignited and weighed. The sesquioxides can be obtained by difference or directly determined in the filtrate by the method already described in I (a).

(E.) *The effect of temperature.* In both the pipette and sedimentation methods care must be taken to avoid temperature fluctuations which create convection currents in the suspension. Further, the viscosity of water has an appreciable temperature coefficient, which must be taken into account in fixing the depth of sedimentation or the depth of pipette sampling. If at 20° C. the depth of sampling is  $h$ , the appropriate depth at temperature  $T$  is given by  $Kh$  where  $K$  has the values given in the following table:—

$T$ . . .	5	10	15	20	25	30
$K$ . . .	0.660	0.784	0.880	1.000	1.125	1.257

(F.) *Change of sampling depth and time.* In the pipette method, if the time fixed for any given sampling is inconvenient, or is missed for any reason, it is only necessary to fix on a new time of sampling, and to adjust the pipette accordingly. Thus in the clay sampling, 7 hours and 8.75 cms. or 10 hours and 12.5 cms. are both equivalent to the usual 8 hours and 10 cms. sampling. It is not advisable to sample at a less depth than 7 cms.

(G.) *The use of summation curves.* Attention should be drawn to the method of reporting the results by a summation curve (Robinson, *J. Agric. Sci.*, Vol. 14 (1924), p. 626), instead of a series of numbers giving the percentage amount of each fraction present. The curve is obtained by plotting the logarithms of the settling velocities employed in the separation against the actual experimental data obtained in the pipette method, i.e. against the weight (or percentages) of clay, clay + silt, etc. The curve thus obtained shows at a glance the characteristic particle size distribution of the soil, and has the further advantage that it is easy by interpolation (and to some extent by extrapolation) to express the results in any other scale for which the settling velocities are known.

#### (H.) *Apparatus for mechanical analysis.*

*The end-over-end shaker.* A convenient end-over-end shaker can be constructed by building a set of wooden compartments on either side of an old bicycle wheel, fitted with a long axle. A small hot-air engine provides an adequate drive.

*The 0.2 m.m. mesh sieve.* This sieve has been usually referred to as the 100-mesh sieve, and there has been some confusion in the past as to the actual dimensions of the holes. Standard specifications of sieve dimensions have been issued by the Institute of Mining and Metallurgy. The I.M.M. sieve, No. 70, with a square hole of 0.18 m.m. side, is the most suitable, as the slight wear and stretch in use brings the aperture dimensions closer to the 0.2 m.m. value. When passing soil through these sieves only a gentle rubbing action should be used, otherwise appreciable enlargements of the apertures may result. (See Keen and Haines, *J. Agric. Sci.*, Vol. 13 (1923), p. 467.)

*The special pipette and stand.* The stand is provided with levelling screws, and embodies a simple wheel and ratchet device by which the pipette can be smoothly raised or lowered to any desired position along a vertical centimetre scale. It is so constructed that it can be easily moved along the bench and placed in turn over a number of cylinders without disturbing them. The pipette has a long stem below the bulb, thus obviating the necessity for wide-mouthed cylinders, and a two-way tap above, so placed that 20 c.c. of suspension are delivered. It is lowered into the suspension to the desired depth with the tap closed. At the sampling time the tap is given a quarter turn and the suspension drawn up by suction until it fills the bore of the tap and has passed a short distance above. A further quarter turn closes the tap, and the pipette is then withdrawn. A final quarter turn is then given, and the suspension in the pipette discharges into the tared dish. The surplus suspension above the tap is restored to the cylinder. The complete apparatus is now on the market.

*The drying oven.* The ordinary water oven has been commonly employed for drying the soil samples and fractions. It is not completely satisfactory, as

the temperature varies from point to point inside and, except possibly on the bottom, rarely exceeds  $95^{\circ}$ – $98^{\circ}$  C. Further, adequate ventilation is often overlooked. A well-lagged electric oven with an adjustable temperature-control device has been found very suitable. The use of a hot-air oven is not recommended owing to the difficulty of temperature control. The water oven can be improved by the addition of some soluble salt or glycerine to raise the boiling temperature of the water to  $105^{\circ}$  C., together with a condenser to prevent evaporation. In any case, the flame should be adjusted so that the water boils vigorously.

## NOMOGRAPHS FOR USE IN MECHANICAL ANALYSIS CALCULATIONS

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### INTRODUCTION

Although the importance of a temperature correction for the viscosity of water in mechanical analysis has been repeatedly emphasized, the necessary corrections are not in general use. Until recently, standard settling velocities have been defined without reference to a standard temperature, and even in coöperative tests on the methods of mechanical analysis there has been no uniformity in this matter. Neglect of temperature effects has been justified on the grounds that no great accuracy is claimed for the results of mechanical analysis and that the methods must be sufficiently simple and rapid for use by routine workers. It is suggested that Nomograph A, described below, disposes of the second objection since the corrected values may be read off directly by setting a straight edge across three scales. Recent developments in methods of pre-treatment and of sampling have so greatly increased the accuracy of mechanical analysis that extremely close agreement is obtained in duplicate determinations carried out together. If such satisfactory agreement is to be obtained on different occasions or in other laboratories or countries, temperature corrections will be needed. Such corrections over a normal temperature range will cause only slight displacements of a distribution curve and will make relatively little change when the curve has been fixed by many experimental points. In the more usual case with only three or four points, the changes produced in the individual fractions will be small if the summation percentage curve is almost straight or only slightly curved. But where any one particle size predominates, i.e. where there is a steep portion on the usual summation percentage curve, the small displacement caused by a temperature difference will make a large difference in the amounts of the two fractions divided by a sampling at a point on this steep portion. Such a difference would greatly distort the distribution curve. An illustration of this point is afforded by determinations by the pipette method of the silt and finer fractions at 10 and 20°. The results differed by 4 per cent for a Rothamsted soil, but by 27 per cent for a separated soil fraction; good agreement was obtained in both cases when the corrected settling velocities were used. Again, in the pipette method of sampling the temperature correction is perhaps more necessary than in the older methods of repeated sedimen-

tation, for in the latter case the effect of any abnormally high or low temperature is smoothed out by the further sedimentations. With a single sedimentation there is a possibility of an occasional wide divergence from the normal laboratory temperature.

### TEMPERATURE CORRECTIONS

Whatever conclusions are reached as to the connection between particle size and settling velocities, there can be no doubt that the temperature correction must depend on Stokes' Law to the extent that in all determinations the product of the velocity and viscosity should be equal to that given by the standard settling velocity at the standard temperature. In what follows Hoskin's data<sup>1</sup> for the viscosity of water have been employed. The standard temperature is taken at 20° C. as recommended at the Rothamsted Conference of the First Commission.

Table 1 gives the factors by which times or depths of sampling at 20° C. must be multiplied so as to give the equivalent times or depths at some

TABLE 1.—Factors for converting times or depths of sampling into other equivalents

t° C.	Time	Depth
5	1.513	0.661
10	1.303	0.767
15	1.136	0.881
20	1.000	1.000
25	0.888	1.127
30	0.795	1.257
35	0.719	1.392

other temperature t° C. Attention may be directed again to the magnitude of these effects. At 10; 20; and 30° respectively, equivalent depths are 7.7; 10.0; 12.6 cm. respectively, and equivalent times for the proposed international fractions,  $9\frac{3}{4}$ ;  $7\frac{1}{2}$ ; 6 minutes; and 10 hours, 25 minutes; 8 hours; 6 hours, 20 minutes respectively. The correction for a few degrees temperature difference is of the same order of importance as a centimeter, a minute or an hour in the three cases illustrated.

Table 1 may be readily extended to give the depths and times for the standard separations at a series of temperatures. It is, however, more convenient to use a nomograph or alignment chart, such as that given in Fig. 1. From this nomograph the corrected values of depth or time at any temperature may be read off immediately, not only for standard values but for all values of depth and time. This is especially useful in connection with the pipette method where it is often convenient to vary

<sup>1</sup> Phil. Mag. 1909, 1: 502; 2: 260.

the values of depth and time whilst maintaining the standard velocity, and especially where it is desired to explore the distribution curve in greater detail by determinations at settling velocities other than the standard ones.

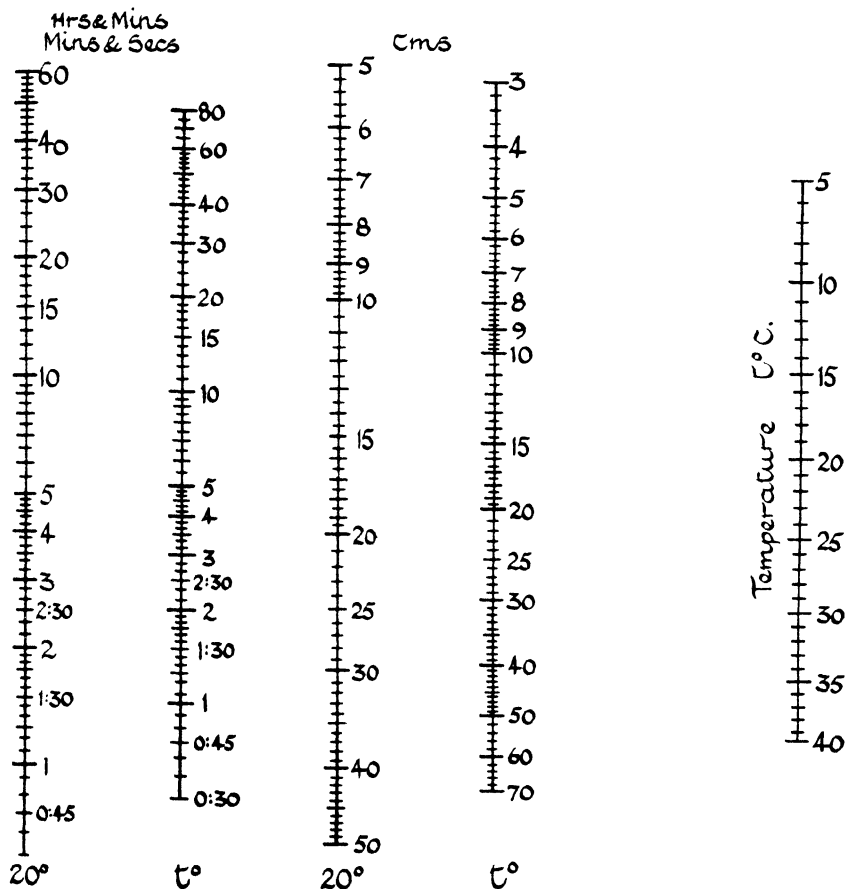


FIGURE 1.—Nomograph A

### DESCRIPTION OF NOMOGRAPH A

Nomograph A in Fig. 1 provides for the conversion of any depth (from 5 to 50 cm.) and of any time (from 35 sec. or min. to 60 min. or hr.) at the standard temperature 20° C. into the equivalent values at any temperature from 5 to 40° C. It should be noted that minutes and hours are divided, not into tenths, but into twelfths (5 sec. or 5 min.) up to 2 minutes (or hr.) and into sixths (10 sec. or 10 min.) from 2 to 5 minutes (or hr.). The nomograph is extremely simple and rapid in use. A straight line joining any depth (or time) on the scales marked

20° with the desired value on the temperature scale cuts the depth (or time) scales marked  $t^\circ$  at the equivalent value of depth (or time) at this temperature.

The straight line may be obtained by a good ruler or a piece of stretched black cotton but most conveniently by a stout celluloid strip with a black line engraved on its lower surface.

#### UNITS IN MECHANICAL ANALYSIS

Other calculations frequently employed in connection with mechanical analysis may be obtained by direct readings from a suitable nomograph. It is becoming increasingly common to express the results of mechanical analysis in the form of summation percentage curves, using as one axis the negative logarithm of the settling velocity ( $-\log v$  with  $v$  as cm. per sec.).

Again it is often desired to express the results in terms of particle size. Several courses are open in the conversion of settling velocity into particle size. Arbitrary but agreed values may be adopted such as those recommended by the First Commission in its conference at Rothamsted in 1926. Alternatively, each worker may make microscopical measurements on a particular group of soils. Differences in the shape of particles would probably necessitate a separate series of values for each worker and for each group of soils, whilst the method obviously breaks down for the finest particles. The third alternative is to use the "equivalent diameters" deduced from Stokes' Law on the assumption of spherical particles of uniform and constant density (the value 2.7 is assumed below).

For popular presentation of the data from simple routine analysis, the first method is probably the best, whilst the second method may be required occasionally for special investigations. The equivalent diameter is free from any ambiguity of meaning and has been widely used in colloid chemistry.

The use of the settling velocity (or  $-\log v$ ) alone is open to the objection that it is not always clear whether the velocities have been adjusted so as to refer to a standard temperature. The product of velocity and viscosity or ( $-\log v\eta$ ) would avoid such uncertainty, but this quantity has peculiar dimensions. A simple function of this product gives the equivalent diameter, a quantity which is readily visualized.

#### DESCRIPTION OF NOMOGRAPH B

Nomograph B in Fig. 2 provides for the direct conversion by a single setting of the rule of depths (from 4 to 80 cm.) and times (from 1 min. to 500 hr.) into the corresponding values of  $-\log v$  (from 0 to 5.5). For any value of  $-\log v$  the required value of depth (or time) can be read off at once for any desired value of time (or depth). This is convenient

for obtaining points between the standard values or for the extremely small settling velocities. For a fixed value of depth it is possible to read off directly a series of values of time giving equal increments in  $-\log v$ . This simplifies the mathematical analysis of data from continuous

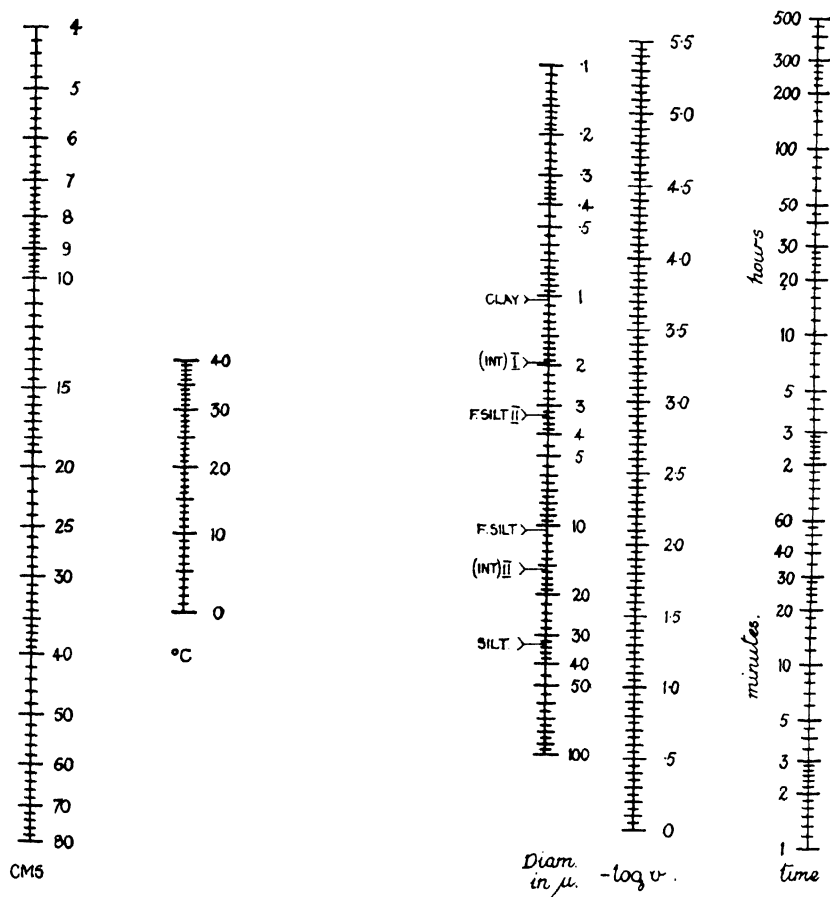


FIGURE 2.—Nomograph B

mechanical analysis methods such as with Wiegner's apparatus or with the one described by the writer in another paper to this Congress.

The three middle lines of Nomograph B provide for the conversion of values of  $-\log v$  and temperature (from 0 to 40° C.) into the equivalent diameter in  $\mu$  (from 0.1 to 100). For convenience the equivalent diameters corresponding to fractions I and II on the proposed international scale, and the fractions Clay, Fine Silt II, Fine Silt I, and Silt on the British scale are marked. It should be noted that these equivalent

diameters differ rather widely from the particle sizes conventionally assigned to these settling velocities.

One setting of the rule converts depth and time into  $-\log v$ , and a second setting through this value of  $-\log v$  and the temperature gives the equivalent diameter. This process may be inverted so as to convert this equivalent diameter into  $-\log v$  at some other temperature and thus a new series of depth and time values. As an illustration a line through depth = 10 cm., time = 8 hr. gives  $-\log v = 3.46$ ; a line through  $-\log v = 3.46$  and  $t^{\circ} \text{C.} = 20$  gives the equivalent diameter =  $1.95 \mu$ . (This value is marked Int. I). Suppose it were desired to take an equivalent sample at  $10^{\circ} \text{C.}$ , then the line through  $10^{\circ} \text{C.}$  and  $1.95 \mu$  gives  $-\log v = 3.57$ , and a further setting of the rule gives for 10 cm. a time of 10.3 hours. If it were necessary to sample at a different time, say 6 hours, then the line through 6 hours, and  $-\log v = 3.57$ , gives as the required depth 5.8 cm.

For the simple temperature correction of a known settling velocity Nomograph A is to be preferred as it is both simpler and more accurate. Nomograph B is intended primarily for the convenient interchange of units.

It has been assumed that the temperature remains constant during the sedimentation. Appreciable temperature changes may produce large errors through complex convection currents. Where some fluctuation of temperature cannot be avoided, the mean temperature (or the mean of the maximum and minimum) should be used for purposes of temperature correction.

It is hoped to be able to publish copies of the Nomographs A and B in forms more suitable for withstanding laboratory usage.





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## STUDIES IN SOIL CULTIVATION. IV.

### A NEW FORM OF TRACTION DYNAMOMETER.

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(With Two Text-figures and Plates XII and XIII.)

#### INTRODUCTION.

IN the first paper of this series<sup>(1)</sup> a description was given of the Watson dynamometer, which was employed for certain developments in the application of dynamometer technique to problems of soil cultivation, described in the second and third papers<sup>(2)</sup>. For the continuation and extension of the experiments to different types of work it became very necessary to employ a dynamometer more adaptable than the original. This was designed for tractor ploughing, when the line of draught between tractor and plough was close to and fairly parallel with the ground surface. The higher and sloping line of draught employed in horse traction rendered the Watson dynamometer useless for extension to this kind of work, and in any case it was much too heavy for the purpose. It was also desirable to improve the method of recording the draught, which required constant and careful attention, especially in adverse conditions. A new dynamometer evolved to overcome these objections is described in the present paper. It is very light and portable, but of robust construction, and by a simple adjustment can be made to cover all ranges of draught from the heaviest to the lightest with the same percentage accuracy. The number of moving parts is very small and adjustments for stylus pressure, etc.—an inconvenient and frequent necessity with other instruments—are very rarely necessary. The system of recording employed is quite unaffected by weather or soil conditions. The apparatus has been very fully tested, and has been found very satisfactory. Its adaptability for different types of work is shown by its use at Rothamsted for horse ploughing investigations and, in India, for heavy mole drainage trials. It has been placed on the market by the Cambridge Instrument Company.

#### DESCRIPTION OF THE DYNAMOMETER.

The dynamometer consists of three main parts: (a) a hydraulic link, in the form of a piston and cylinder containing oil, placed in the hitch of the implement; (b) the mechanism that records the fluctuations of

pressure on the oil as the implement is drawn forward; (c) control box, carried by the operator.

Copper tubing containing oil is used to transmit the pressure in the hydraulic link to the recording mechanism. The tubing is coiled in a spiral, so that alterations in the relative positions of link and recorder during work can occur without any strain on the apparatus. This arrangement also allows the recorder to be disposed in any convenient position.

The general arrangement of the constituent parts is shown in Pl. XII, fig. 1, and diagrammatically in Fig. 1. Referring to the figure, the hydraulic link *A*, is shown on the left. No serious disturbance is produced in the

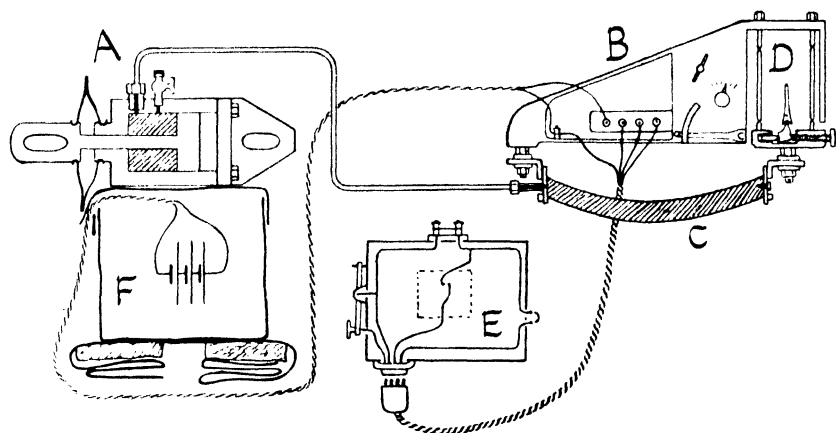


Fig. 1. The Rothamsted dynamometer in diagrammatic form.

line of draught when this is introduced into the hitch of an implement as its weight is only 16 lb., and the extra length entailed (about one foot) can, if necessary, be compensated by removing a few links from the hitch chain. A hollow leather collar is fixed over the end of the cylinder and the protruding portion of the piston, to prevent the entry of dust and water. The pressure is transmitted through the oil in the flexible copper tubing (not shown coiled in the diagram) to a Bourdon tube (C) also containing oil, and bent into the arc of a circle. The variations in oil pressure produce changes in the curvature of the arc, and in consequence, alterations in length of the chord. This change of length is very small, and is therefore directly proportional to the oil pressure, and thus to the soil resistance. The change of length is magnified and recorded by the mechanism *B* which is the well-known Stress Recorder manufactured by the Cambridge Instrument Company. The Bourdon tube is

rigidly bolted at its left-hand end to the frame of the Recorder, while the right-hand end is bolted to a floating stage. Three interchangeable Bourdon tubes are provided, of different strengths for use in light, medium and heavy work, respectively. The floating stage is supported by three steel pillars whose diameter is reduced at the points shown, to allow the stage to move to and fro in response to the extension and contraction of the Bourdon tube. This movement is magnified and transmitted to the recording arm *D* by a simple and robust system of two knife-edge levers. Attached to the upper portion of the arm is a very small rounded stylus, pressing against a celluloid ribbon 11 mm. wide that is advanced continuously by clockwork within the recorder casing. The speed of the clock can be varied within wide limits by turning an indicator on the recorder, and it can be started or stopped by an electrical control. The stylus does not scratch the celluloid, but causes it to flow plastically, so that the trace is impressed in the form of a groove bounded on each side by a small ridge. The line of zero draught can be adjusted by the small milled-head shown on the right of the floating stage, while the pressure of the stylus on the celluloid is adjusted, if necessary, by a second screw, not shown in Fig. 1. This method of recording has very great advantages, for the trace is permanent, and is unaffected by oil, dirt, or water. If necessary the celluloid ribbon can be cleaned by dipping it in water and wiping it dry with a cloth.

The small movement of the floating stage and the simple lever system of magnification result in the movement of the recording stylus being proportional to the stress applied to the hydraulic link. Direct calibration curves connecting stylus position and stress were obtained by suspending the link vertically, adding equal increments of weight up to the maximum, and then reducing the load by equal amounts to zero. The calibration curves for increasing and decreasing stress were linear, and identical.

In addition to the stress-recording stylus there are two others, that operate on the back of the ribbon, to avoid fouling the main stylus. One is used for recording time intervals and the other for position marks, and both are controlled electrically.

The three controlling circuits—clock for chart movements, time-recording clock, and position-recording key—are governed from the control box *E*, weighing only 4 lb., and carried in a light harness by the operator. The box carries (a) a switch for starting or stopping the chart, (b) a clock that momentarily closes, at ten second intervals, the circuit operating the time stylus, and (c) a tapping key for closing the circuit

operating the position stylus. The tapping key is used for recording position marks, passage from one plot to another, etc., and for marking on the chart in the Morse code any other details of importance. This feature is valuable: not only does it eliminate any possibility of confusion when the records of a complicated experiment are examined in the laboratory, but it also serves for recording important notes on the soil conditions, the behaviour of the implements, etc., that would otherwise have to be entered in a notebook while the experiment was actually in progress.

The three circuits have a common return wire, and are brought out to the four terminals of an ordinary wireless valve holder, so that a convenient and automatically correct connection can be made through a four-pin plug to the cables from the recorder. The cables are joined into one strand and lightly armoured for protection. The current for the circuits is provided by a six volt battery *F*, that can be carried in any convenient position. The low internal resistance type of dry battery is quite suitable for the purpose. In Fig. 1 and Pl. XII, fig. 1 they are shown in a leather case, riveted to a surcingle so that they may be carried on the back of the horse. For tractor ploughing the batteries would be carried on the tailboard, in which case they would be more conveniently connected to the two terminals shown on top of the control box.

A direct reading pressure dial gauge can be introduced if required into the oil system at any convenient point, or can be attached to the hydraulic link in place of the copper tubing and the recorder. Two such gauges, for heavy and light work respectively, and calibrated to give directly the pull applied to the hydraulic link, are shown as part of the dynamometer accessories in Pl. XII. Its main use is for purposes of demonstration, or for the securing of preliminary information. The limitations of a direct reading gauge as compared with a recording device are discussed later.

#### ASSEMBLY FOR FIELD WORK.

The method of assembly for field work is as follows. The hydraulic link is connected into the hitch, and the stress recorder, which weighs only 15 lb., is strapped in any convenient position on the implement, *e.g.* the beam of a plough, or the frame of a cultivator, care being taken to pad it with a roll of felt against mechanical shock, since the stylus responds to such vibrations. The Bourdon tube must also be unrestricted, and not liable to any casual contact with the implement. The link and the recorder are then connected by the spiral of copper tubing. This is

provided in fairly short lengths with screw unions so that it can be easily adapted to varying dispositions of the link and recorder. It is also necessary to tie down the tubing to some support at a short distance from each of the two ends, so that strains produced when turning at the headlands are not concentrated at these points but properly taken up in the spiral part of the tubing.

The whole system is then filled with oil from an oil-gun. The batteries are mounted, and if necessary the cables tied with string to suitable points to prevent them from dragging on the ground or fouling the implement. The operator then straps on the control box, inserts the four-pin plug into the socket and gives the signal for work to begin.

A photograph of the dynamometer in use for horse ploughing is reproduced in Pl. XII, fig. 2.

The operator walks beside the implement, using the tapping key to record on the chart the points of passage past pre-arranged points. For the general work, these points are previously fixed by stakes placed in the ground at suitable places. The tapping key is also used, as previously mentioned, for Morse code notes of the work.

The celluloid ribbon passes out through a slot in the recorder, and convenient lengths of it can be cut off at intervals. A low power microscope is provided in the kit, so that the record can be examined in detail to check the correct functioning of the recorder. The portability of the complete equipment may be judged from Pl. XIII, showing it packed for transit, in a strong oak box, of outside dimensions  $21 \times 15 \times 14$  in. The total weight, including spare oil, tool bag (carried in the box but not shown in the photograph) is under 100 lb.

#### LABORATORY EXAMINATION OF THE RECORDS.

The examination and reduction of the records is done in the laboratory. The trace is too small for direct integration, so this process must be carried out on enlarged copies. The optical properties of the groove and flanking ridges that constitute the actual trace on the celluloid give rise to a very clear and sharp magnified image, that can be taken on sensitive paper, or copied by hand. In many uses of the Stress Recorder, requiring an intensive examination of the stresses during a short interval, the photographic method of enlargement has been found best. But the nature of the problem in soil resistance measurements is quite different. Although minor fluctuations in resistance can be recorded with great fidelity, their exact significance refers primarily to the occurrence of stones in the soil, to the gait of the horses and other casual factors,

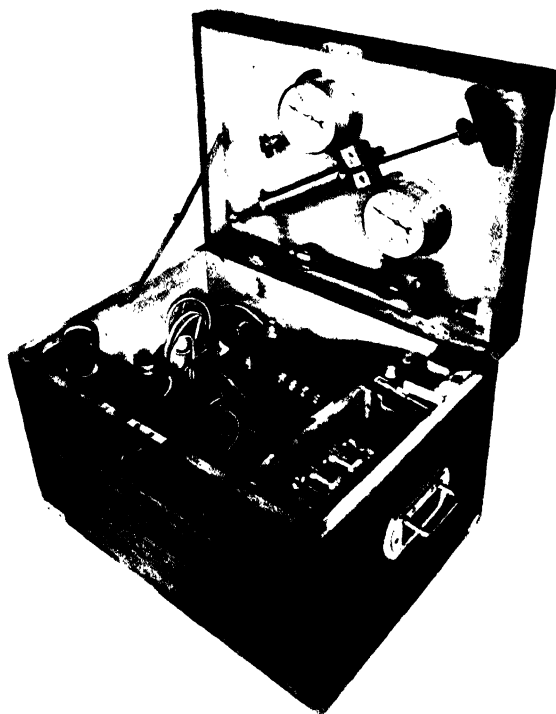


Fig. 1. The Rothamsted dynamometer, showing the units assembled.



Fig. 2. The dynamometer as arranged for horse-ploughing measurements.





Showing dynamometer and accessories packed for transport. Total weight less than 100 lb.



whereas the present interest of the results for soil research lies in the mean values of soil resistance obtained from extended records.

The method we have adopted is to use a low power microscope and camera lucida to transfer an enlarged image ( $\times 34$ ) of the trace to paper, after which it can be divided into its sections as shown by the position marks, and the mean stress of each section calculated by integrating the area with a planimeter, and applying the linear calibration factor. The chart is fed, a step at a time, between two glass plates, mounted a short distance apart, that serve to keep it flat and retain it in position.

With the ribbon speed employed in horse work it is possible to get two adjacent sections of the trace, each representing about twenty-two yards of ploughing, into the field of view at the same time.

#### THE CHARACTER OF THE RECORD UNDER DIFFERENT CONDITIONS.

The whole question of the amount of detail to record in the stress fluctuations either on the trace itself, or in the enlarged copy, requires some judgment to obtain the best results. The instrument is capable of recording stress fluctuations in full detail, if filled with thin oil, and run with a chart speed high enough to open out the trace. Conversely the fluctuations in the record may be smoothed or damped out to any required extent by the use of thick oil, and by introducing a needle valve constriction in the copper tubing. Damping, however, makes the instrument sluggish, and when it was carried to the point of smoothing out all minor fluctuations, the time lag introduced between the application of the stress at the link and the complete response of the recorder was found to be twenty seconds. For horse work this corresponds to a distance of twenty yards or more, and the position marks impressed on the chart would refer, not to the part of the soil resistance trace immediately above them, but to a part some indefinite distance along the trace.

The details of the trace are also obscured if the record is condensed by using a slow chart speed, yet a reasonably condensed record is desirable for enlargement, since the field of the microscope then covers a good portion of the work. With a little experience a good compromise between these opposing requirements can be secured. In general a chart speed of about  $1/4000$  that of the implement gives good results. This is equivalent to one yard of chart for each two and a half miles travel of the implement. In horse ploughing this relation implies a chart speed of about 0.6 in. or 15 mm. per minute. With tractor or steam power implements, a faster chart speed is needed. For the dimensions of the copper tubing usually employed (length about 150 cm., internal diameter

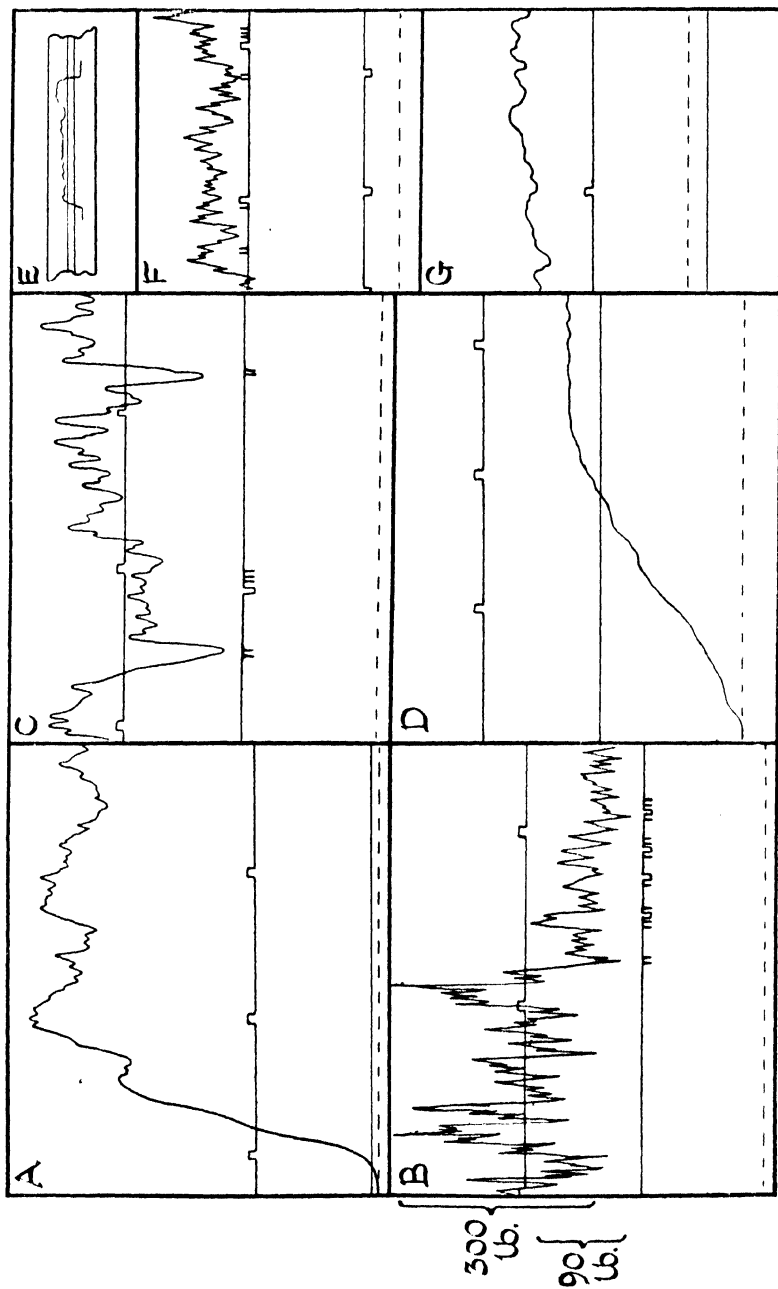


Fig. 2. Enlarged facsimiles of different types of trace; horse ploughing.

4 mm.), an ordinary engine lubricating oil is quite suitable for the pressure system in winter, but it is desirable to thicken it with gear oil in the warmer months.

The above remarks are illustrated by Fig. 2, showing different types of trace obtained with a horse plough. They are facsimile reproductions obtained with microscope and camera lucida as already described. As reproduced in Fig. 2 the magnification is about 10 diameters; a piece of the original trace is reproduced at *E* to the same scale, for comparison. In each of the enlarged traces the dotted line shows the position of the line of zero draught. The two straight full lines are the time and position scales.

The effect of damping is shown in a comparison of traces *A*, *B* and *D*, that refer respectively to normal, insufficient, and excessive damping. Trace *B* was obtained with thin oil and in warm weather; the record is characterised by many sharp changes in direction of the stylus movement, as it follows the abrupt changes in soil resistance. Trace *A* shows a normal amount of damping. The amplitude of the short period fluctuations is somewhat reduced and the sharp points are now somewhat rounded. A trace of this type is much easier to integrate with a planimeter than trace *B*, while the contribution to the total area of the areas within the sharp peaks is so small that their omission is unimportant. The time lag corresponding to trace *A* is not too large, as may be seen by the quick rise of the trace from the zero-draught line when the plough enters its work. Trace *D* shows excessive damping. The minor fluctuations have largely disappeared, and the trace takes about 20 sec. (two marks on the time scale) to rise to its full value.

The effect of the type of work itself on the character of the record is illustrated in traces *F*, *G*, *C* and *B*.

Trace *F* is a typical record on Rothamsted soil, a heavy loam with numerous flints, and shows the jerky "harsh" nature of the pull; trace *G* shows the much smoother pull characteristic of the Woburn sandy soil. Trace *C* is a record of work on Rothamsted soil with a ridging plough that was used in the early spring to break up old weathered furrows, containing much turf. It shows large fluctuations due to the uneven nature of the work, but the record is much more rounded than that in trace *F*. This is due to masses of turf acting as semi-plastic pads or buffers to the motion of the implement through the soil, and in consequence smoothing out the rapid fluctuations in pull. Trace *C* also demonstrates the use of the position marks for the identification of particular portions of the record. Two such marks (each consisting of a double dot) are shown, specifying the beginning and end of plot *B*, that

is indicated in the Morse code (- · · ·). The position marks referred to the passage across the open furrows of the former ploughing; the drop in draught as the plough crosses these trenches is well shown, and the absence of any time lag is also demonstrated.

Trace *B* further illustrates the relation between the nature of the work and the type of record. The record falls obviously into two sections separated by the position mark (· ·), that represents the boundary between a stubble badly infested with couch grass (on the left), and a fallow, well cultivated portion (on the right). The latter is marked in Morse code with the first four letters of the word "fallow." On the stubble section the matted grass and clods of soil obstructed the plough, causing wide and erratic fluctuations in pull, covering a range of about 300 lb., which, by reference to the zero-draught line, is seen to represent over half the average value. On passing to the fallow portion, the implement travels normally, and the fluctuations at once settle down to the much smaller range of about 90 lb.

#### THE LIMITATIONS OF A DIRECT-READING DYNAMOMETER.

The question whether the addition of a recording instrument could be avoided by taking the readings direct from the pressure-gauge attachment, can now be considered in the light of the above discussion.

It is evident, for the type of investigation dealt with in this series of papers, that it would be quite impossible to take accurate readings from a pointer that is rapidly fluctuating over half its total range, and even if this were possible the readings could not be taken down rapidly enough to secure a good mean for small-sized plots. Quite apart from this, the noting of position marks, speed of work, and other details would necessitate at least one extra observer and very practised and efficient co-operation if the results were to be reliable. Any method of damping the fluctuations sufficiently to make direct reading possible, quite spoils the coincidence between recorded draw bar pull and field position. There appears no escape from the conclusion that integrated records of a self-recording instrument are essential for reliable results, although for simple field demonstrations over a large area, a direct reading implement with heavy damping can usefully be employed.

#### SUMMARY.

A new and improved type of dynamometer is described which by a simple interchange of parts can be used with the same percentage accuracy for all types of work from the lightest to the heaviest.

The instrument consists of (a) an hydraulic link weighing 16 lb. and placed in the hitch, (b) a recording mechanism weighing 15 lb. carried on any convenient part of the implement, and (c) a control box weighing 4 lb. carried by the operator. When packed in a stout box for transit and with all accessories the total weight is less than 100 lb. The instrument is of robust construction and has a minimum number of moving parts. Adjustments for stylus pressure, etc., are provided, but the necessity for using them hardly ever arises.

The instrument operates by recording the amount of movement in a Bourdon tube filled with oil and connected by narrow bore copper tubing to the oil in the hydraulic link.

The recording mechanism has been adapted from the Cambridge Instrument Company's Stress Recorder. The trace is impressed on a narrow, moving celluloid ribbon, and is permanent, and also unaffected by water, oil, or dirt. The optical properties of the trace give a clear and sharp magnified image, which can be traced by hand or photographed for purposes of integration. The construction of the instrument is such that the movement of the recording stylus is directly proportional to the stress applied to the hydraulic link.

In addition to this record of draught two other records, operated electromagnetically, are impressed on the ribbon: (a) a time trace showing a mark for each ten seconds, and (b) a position trace on which the passage from one plot to another is marked, and on which any field notes or other details are impressed in the Morse code by means of a tapping key carried by the operator. The styluses for these two records operate on the back of the ribbon so that the motion of the stylus recording the draught is unobstructed.

The method of magnifying the charts for integration with a planimeter is described, and typical records are reproduced, showing the character of the trace for different types of work, and the relation between the amount of detail on the trace and the degree of damping introduced into the oil system.

#### REFERENCES.

- (1) KEEN and HAINES (1925). *J. Agric. Sci.* 15, 375-386. (Rothamsted Memoirs, 12.)
- (2) HAINES and KEEN (1925). *J. Agric. Sci.* 15, 387-394; 395-406. (Rothamsted Memoirs, 12.)

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# "SINGLE VALUE" SOIL PROPERTIES: A STUDY OF THE SIGNIFICANCE OF CERTAIN SOIL CONSTANTS.

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(With Four Text-figures.)

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## 1. INTRODUCTION.

ATTEMPTS have been made by many workers to assess the general character of a soil by measuring one property (or one group of properties) thus specifying the soil by a single number, in place of the group of figures obtained from a detailed mechanical analysis. Such measurements will be referred to in this paper as "single value" determinations.

At present there is no accepted standard physical specification of soil except its mechanical analysis by some recognised method, and this is not a single value function. Although useful information can be extracted from the mechanical analysis as to the probable field behaviour of the soil and its general characteristics, the conclusions are not quantitative or absolute: a good deal of personal judgment, based on previous experience, must be exercised in the interpretations, and, to add to the difficulty, many so-called standard methods do not secure complete disintegration of the soil aggregates. The use of hydrogen peroxide, introduced by Robinson<sup>(1)</sup>, and now adopted in the British<sup>(2)</sup> and International methods<sup>(3)</sup>, has removed the latter difficulty, but Odén's<sup>(4)</sup> noteworthy attempt to express mechanical composition as a continuous function of particle size has not, as yet, overcome the serious inherent

experimental error detected by Coutts and Crowther(5). It is therefore still necessary to express the results of a mechanical analysis as a limited number of groups of particles having specified diameter limits, and this is a crude and unsatisfactory basis for a standard physical specification.

Numerous attempts have been made to use some single value basis of specification. They have been based either on the measurement of moisture content or moisture relationships under defined experimental conditions, or on the colloidal properties of the soil. The hygroscopic coefficient, moisture equivalent, and wilting coefficient, due largely to Briggs and his associates, are among the well-known examples of the former class whose significance has already been discussed by one of us (6). To these may be added, as typical later examples, the rate of evaporation at a given moisture content(7), and the so-called "suction-force" of the soil for water(8).

Measurements depending more or less on the colloidal properties of the soil are its power of dye absorption(9), the heat of wetting(10), the shrinkage of a kneaded block of moist soil(11), and the "sticky point," defined as the moisture content at which kneaded moist soil just ceases to adhere to external objects(12).

There are objections to the use of some of the above determinations as single value constants.

The hygroscopic coefficient is an unsound conception, and the values customarily reported are not equilibrium values at all(13). The wilting coefficient was shown by the work of Alway(14) and Shull(15) to be a complex determination into which the soil, the plant, and the atmospheric conditions all enter.

In spite of certain empirical details the moisture equivalent has attractions as a single value measurement; Cameron and Gallagher(16) showed that it was approximately equal to the practical "optimum moisture content" for plant growth, and more recently Shaw(17) has shown that the equilibrium moisture content in irrigated soil where no ground water table exists is also equal to the moisture equivalent. The fact that few soil laboratories can afford the cost of the apparatus led us to exclude this determination from our present investigation.

The rate of evaporation at a given moisture content is not a suitable measurement, for recent critical examination(18) shows that extreme care is required to obtain reproducible results, and in any case they are difficult to interpret. The work of Haines(19) and R. A. Fisher(20) has elucidated the true nature of the so-called "suction-force," and it is evident that it will not serve for our present purpose.

As originally introduced, measurement of dye absorption was based on the theory of a simple physical adsorption, with the idea that comparisons of effective particle surface between different soils would be possible; this concept has long since been abandoned. The same idea is implicit in the heat of wetting determination; Bouyoucos (10 *b*) and other workers have devoted much attention to this measurement, which has some attractive features. However, the results of some work at Rothamsted, at present unpublished, led us to exclude it, for the time being, from our list of suitable methods.

The shrinkage of a plastic block, and the sticky point measurement were included in our investigations and are discussed below.

## 2. SCOPE OF THE PRESENT INVESTIGATION.

The single values selected for study were those that could be measured with simple apparatus, and which were either known to be measuring some definite soil characteristic or seemed to offer promise of doing so.

The determinations made were as follows:

Percentage of clay fraction<sup>1</sup> (*C*).

Moisture content of air-dry soil (*A*).

Moisture content in equilibrium with atmosphere of 50 per cent. relative humidity (*R*).

Loss on ignition of dry soil (*I*).

Moisture content at sticky point (*S*).

Haines' method for volume shrinkage (11 *c*) was also used to obtain the following derived quantities:

Moisture content of saturated block (*m*).

Pore space of oven-dried block (*P*).

Apparent specific gravity of block ( $\sigma$ ).

True specific gravity of block ( $\rho$ ).

Where necessary the suffixes *o* and *p* are used to distinguish between values for original soils and those treated with hydrogen peroxide as described immediately below.

The percentage of calcium carbonate in the soils was also determined.

A number of other measurements were made which are referred to in the body of the paper.

<sup>1</sup> The experiments were completed before the Revised British Official Method of Mechanical Analysis (*Agricultural Progress* (2 *c*)) was adopted. The clay figures are those obtained by the earlier method (*Agricultural Progress* (2 *b*)). The values for the clay content given in this paper are therefore less than they would have been with the new method, but the differences are relatively small and do not appreciably affect any of the conclusions that have been drawn.

The most important feature of the present investigation, apart from the careful selection of suitable single value determinations, was the repetition of the measurements after the soils had been treated with hydrogen peroxide. It is evident that both organic matter and the mineral part of the soil are concerned to varying degrees in all single value measurements, and until recently there has been no experimental means of separately assessing the effects. For this purpose the use of hydrogen peroxide appeared well worthy of extended trial, since, in any single value determination, the difference in results for the original soil and the peroxide treated soil would give a direct indication of the contribution of the organic matter to the value. This implies, however, that the peroxide is without effect on the mineral portion of the soil. The assumption is not strictly true: a small amount of mineral material is dissolved that comes principally from the clay fraction and consists of mixed sesquioxide and a small quantity of silica (2a). It is not yet definitely known whether the removal of this material appreciably alters the physical and physico-chemical properties of the mineral portion of the soil, but the available evidence is rather against this possibility.

In the present investigations, therefore, it has been assumed that peroxide has removed humified organic material without affecting the physical properties of the mineral part of the soil.

### 3. THE SOILS USED.

In all, 39 soils were subjected to the complete set of measurements outlined above, both on original and peroxide treated samples. The soils selected showed a wide range of type and properties, varying from heavy clays to light sands, a number of samples from depths below the top soil were also included, together with certain Rothamsted and Woburn soils that differed widely in manurial treatment.

Prior to the experiments all the soils were air dried and passed through a 1 mm. sieve with round holes.

Table I gives a descriptive list of the soils employed, which are top soils unless otherwise stated.

### 4. DESCRIPTION OF METHODS.

*Percentage of clay (C).* This was determined on the old basis for this fraction (settling velocity 8.6 cm. in 24 hours at 15° C.) in the usual way.

*Air-dry moisture content (A), moisture at 50 per cent. relative humidity (R) and loss on ignition (I).* The air-dry moisture content was determined in the ordinary way, the soil being heated 24 hours in a steam oven.

Table I. *List of soils used.*

Soil No.	Locality	Type
1	Cwybr Fawr, Rhyl, Wales, F. 86	Sandy loam, Triassic drift
2	Chwaen Goch, Llanerchymedd, Anglesey, Wales, A. 132	Silt loam, Ordovician shales
3	Garforth, Yorks, 34	Clay loam, Coal measures
4	Garforth, Yorks, 113	Light loam, Coal measures
5	Woburn, Stackyard Field. Unmanured	Light sand, Lower greensand
6	Woburn, Stackyard Field. Farmyard manure	Light sand, Lower greensand
7	Rothamsted, Hoosfield Fallow. 9-18 in.	Heavy loam. Subsoil, Clay with flints
8	Rothamsted, Hoosfield Wheat. 9-13½ in.	Heavy loam. Subsoil, Clay with flints
9	Rothamsted, Barnfield. Farmyard manure. 0-9 in.	Heavy loam. Subsoil, Clay with flints
10	Rothamsted Sawyers Field	Heavy loam. Clay with flints
11	Melchet Court	Sandy soil
12	Craibstone, Aberdeen	Light sand. Limed, Granitic drift
13	Craibstone, Aberdeen	Light sand. Unlimed, Granitic drift
14	Craibstone, Aberdeen	Light sand. Limed subsoil, granitic drift
15	Craibstone, Aberdeen	Light sand. Unlimed subsoil, Granitic drift
16	Vaynol, near Bangor, Wales	Raw subsoil. Heavy Carboniferous Limestone clay
17	Deep excavation in Oxford Street, London	London clay
18	Wad Medani, Sudan	Gezira soil (badobe)
19	Mikveh, Palestine	"Argileuze" (0-30 cm.)
20	Harpندن Common	Clay with flints
21	—	Kaolin (B.D.H. purified)
22	Dagenham, Essex. 107	Recent gravel
23	Dagenham, Essex. 107	Subsoil, recent gravel
24	White Roding, Essex. 72	Boulder clay
25	White Roding, Essex. 72	Subsoil, Boulder clay
26	Abbess Roding, Essex. 73	Boulder clay
27	Abbess Roding, Essex. 73	Subsoil, Boulder clay
28	Thundersley, Essex. 117	Bagshot
29	Thundersley, Essex. 117	Subsoil, Bagshot
30	Rochford, Essex. 127	Brickearth
31	Rochford, Essex. 127	Subsoil, Brickearth
32	Ardleigh, Essex. 162	Glacial loam
33	Ardleigh, Essex. 162	Subsoil, Glacial loam
34	Beaumont-cum-Moze, Essex. 172	Alluvium
35	Beaumont-cum-Moze, Essex. 172	Subsoil, Alluvium
36	St Osyth, Essex. 177	Marsh alluvium
37	St Osyth, Essex. 177	Subsoil, Marsh alluvium
38	Gt Wigborough, Essex. 183	London clay
39	Gt Wigborough, Essex. 183	Subsoil, London clay

The moisture content at 50 per cent. relative humidity was determined as follows:

About 10 gm. of the air-dry soil were placed in a squat weighing bottle and exposed over sulphuric acid (density 1.3325 at 15° C.) in a vacuum desiccator kept in a dark room at approximately constant temperature. Equilibrium was assumed to be reached when the weight did not change by more than 1 mgm. in 2 days. The weighing bottles were then transferred to a second vacuum desiccator containing concentrated sulphuric acid and allowed to reach equilibrium as before.

A portion of this dried soil was transferred to a silica basin, and ignited in the muffle at a bright red heat, cooled and weighed.

The final weighings for the soil after reaching equilibrium in the desiccators are probably accurate to two or three milligrams, and the results are therefore reliable to about 0.02 per cent. The loss on ignition, however, is far less accurate, and there is some uncertainty in the end point even after prolonged ignition at bright red heat. Increasing the period by successive three-hour periods up to 12 hours gave, in most cases, increasing loss on ignition, but with certain soils there was sometimes an increase in weight when the time was lengthened, presumably on account of the oxidation of some residual constituent of the soil. On account of these fluctuations the accuracy of the final figures cannot be higher than about 0.2 per cent.

*Sticky point (S).* Although with practice the recognition of this point becomes an easy matter except for a few soils, the determination requires a certain amount of care if the results of different observers are to be comparable. Tests of this point are discussed separately below in Section 5 (*d*). A large number of experiments were first carried out with a small model mixing machine of the type used for dough and cordite, in which two horizontal shafts furnished with paddles and placed at the base of the machine, revolve inwards at different speeds. This machine effects a very thorough mixing of the soil and added water, but was unsuitable for our purpose because of the quantity of soil required (about 100 gm.) and the inconvenience caused by the dismantling and cleaning of the apparatus between the determinations for each soil. After several methods had been tried the following was adopted as standard: about 10 gm. of soil were spread in a thin layer on a glass plate and distilled water was added from a fine jet until the soil was definitely wet and sticky. The mass was then worked into a paste with a knife spatula. The wooden handled palette knife type with a flexible steel blade about 13 cm. long and  $2\frac{1}{4}$  cm. broad was found very suitable for this purpose. The mass was then scraped from the plate and kneaded by hand until the soil just reached the stage at which it no longer stuck to the hands or the knife. At this stage it was possible to cut cleanly through the plastic mass with the knife. As already mentioned, this point could with a little practice be readily identified<sup>1</sup>.

<sup>1</sup> In the earlier trials with the mixing machine the attainment of this condition was signalled by a perceptible cleaning-up of the metal base and sides of the apparatus, and it was because this observation was less likely to be influenced by personal judgment than the behaviour of the soil in the hands of the experimenter that the machine was tried in the first instance.

A sample of the kneaded soil, after weighing, was dried in the steam oven to determine the moisture content. At least two determinations were made for each soil, and they did not generally differ from each other by more than 1 per cent.

*Determination of derived quantities from volume shrinkage experiments.* The volume shrinkage of the soil was determined by the method described by Haines (11 c). From these data it is possible to calculate the pore space ( $P$ ) of the oven-dried block, and the apparent and true specific gravities ( $\sigma$ ,  $\rho$ ) of the block (either before or after oven drying). An important characteristic of the shrinkage curve, depending upon the type of the soil, is defined by the moisture content ( $m$ ) at which the first reading is taken.

*Peroxide treated soils.* With the exception of the air-dry moisture content, and the mechanical analysis (in which the peroxide and acid treatment were naturally used), the above observations were repeated on the soils after treatment with peroxide. The treatment followed that laid down in the British method of mechanical analysis (2 a), except that no acid was used. The soils were washed, dried at room temperature, and the dried cake was disintegrated with a wooden pestle.

## 5. DISCUSSION OF EXPERIMENTAL RESULTS.

The data obtained are shown in Table II. As a preliminary, scatter diagrams were drawn to obtain some idea of the association between possible pairs of the experimental quantities, and the rough conclusions from these diagrams showed which associations were worth closer examination. It will be convenient to discuss firstly a number of smaller points before entering on the main examination of the data:

The moisture content ( $m$ ) at the highest reading of the shrinkage curve is not a very sharply defined point but it is obviously closely related to the sticky point of the soil, and gives a high correlation with that quantity. Hence the length of the shrinkage curve can most usefully be defined by extrapolation to the latter value. Similarly the values for the air-dry moisture content ( $A$ ) are closely related to the equilibrium moisture content at 50 per cent. relative humidity ( $R$ ). The quantities ( $A$ ) and ( $m$ ) therefore need not be further considered.

The pore space as measured by the shrinkage experiment is not that of the soil in its normal state, but of a well-kneaded block. A measurement of pore space and the related quantities of real and apparent specific gravity in conditions similar to the field state was developed by one of us in an earlier paper (21), working with a series of successive depth

Table II.

Soil No.	Untreated samples										Peroxide treated samples									
	Shrinkage data					Moisture data					Shrinkage data					Moisture data				
	<i>P</i>	$\sigma$	$\rho$	<i>m</i>	<i>A</i>	<i>R</i>	<i>S</i>	<i>C</i>	<i>I</i>	CaCO <sub>3</sub>	<i>P</i>	$\sigma$	$\rho$	<i>R</i>	<i>S</i>	<i>I</i>				
1	26.5	1.83	2.49	21.4	1.99	1.81	34.3	15.2	7.29	0.7	20.6	2.06	2.59	1.62	19.8	4.09				
2	35.0	1.54	2.36	43.3	3.55	3.05	56.7	20.7	13.76	0	21.3	1.97	2.50	1.14	30.0	8.98				
3	27.6	1.76	2.44	36.5	3.51	3.27	46.0	23.9	11.08	tr.	26.7	1.85	2.52	2.38	29.5	6.53				
4	26.7	1.80	2.46	17.7	1.15	1.10	27.3	7.2	5.12	tr.	21.7	1.87	2.38	0.96	22.2	3.04				
5	22.7	1.95	2.60	14.3	1.46	1.30	19.7	7.4	3.60	0	18.6	1.95	2.44	0.98	17.2	2.14				
6	24.8	1.90	2.53	17.4	1.37	1.29	22.3	8.3	4.45	0.021	17.6	2.00	2.42	0.98	17.2	2.14				
7	23.5	1.98	2.59	27.2	3.15	2.93	26.8	21.7	5.27	0	22.1	2.00	2.37	3.17	27.0	4.67				
8	27.3	2.00	2.60	31.0	3.52	3.13	30.1	27.2	6.75	0.180	22.3	2.02	2.60	3.46	29.4	5.08				
9	27.3	1.88	2.58	30.2	5.12	2.59	28.8	21.8	9.13	1.8	22.6	1.98	2.56	2.74	31.4	6.14				
10	28.2	1.85	2.57	26.2	2.29	2.09	29.3	13.9	6.25	0	21.6	1.98	2.52	1.88	21.2	3.73				
11	31.0	1.71	2.48	21.9	1.55	1.45	29.3	8.4	4.75	0.02	23.8	1.84	2.41	0.94	22.0	2.27				
12	37.2	1.58	2.52	30.5	3.39	3.28	44.7	1.4	9.04	0.303	26.5	1.84	2.50	2.13	27.9	5.16				
13	34.3	1.64	2.49	26.4	2.48	2.41	43.1	1.4	9.23	0	24.7	1.79	2.37	1.91	24.8	4.15				
14	35.5	1.62	2.52	24.5	3.56	3.29	36.9	1.9	8.82	tr.	25.8	1.73	2.38	2.74	33.8	7.47				
15	24.8	1.91	2.54	13.5	1.19	1.06	22.2	0.3	3.60	tr.	19.8	1.93	2.40	0.95	20.1	2.07				
16	23.3	1.95	2.61	38.8	6.12	5.26	35.0	52.0	13.09	10.9	21.0	1.96	2.48	5.29	37.2	14.30				
17	23.9	1.94	2.55	40.4	5.80	4.99	38.8	45.0	11.46	1.2	21.5	1.98	2.53	5.39	33.7	6.18				
18	16.5	2.11	2.53	38.3	9.37	8.82	39.1	47.6	8.13	2.13	16.2	2.14	2.56	8.57	40.0	8.65				
19	17.2	2.11	2.55	42.8	7.83	7.17	33.7	40.8	9.42	3.81	15.4	2.14	2.53	7.68	35.7	7.64				
20	34.4	1.63	2.48	43.4	2.89	2.41	45.4	19.8	9.85	tr.	25.2	1.88	2.51	1.76	28.3	4.32				
21	37.2	1.53	2.43	35.9	0.53	0.48	40.6	44.2	12.34	0	38.2	1.52	2.46	0.60	43.5	12.42				
22	25.9	1.77	2.39	20.2	1.88	1.50	29.8	6.6	7.73	0.05	22.0	1.95	2.50	1.35	20.9	3.42				
23	21.4	1.93	2.46	18.7	1.49	1.15	21.4	8.7	4.27	0	19.5	2.02	2.52	1.44	19.5	2.35				
24	23.7	1.88	2.46	31.0	4.06	3.54	37.2	22.9	7.32	0.06	17.2	2.01	2.52	3.29	27.4	4.84				
25	21.0	2.00	2.74	47.4	5.16	4.28	37.0	30.9	6.25	0	19.0	2.02	2.49	4.53	32.0	4.94				
26	21.0	1.96	2.49	27.3	3.53	2.99	33.1	21.7	11.46	11.62	19.2	2.04	2.53	3.33	30.3	11.00				
27	22.5	2.01	2.60	28.5	3.46	2.87	30.8	23.0	14.71	22.80	18.6	2.04	2.50	3.26	29.4	14.27				
28	25.0	1.88	2.51	19.7	2.14	1.87	26.8	10.8	4.92	0	22.5	1.93	2.48	1.73	23.1	2.59				
29	23.4	1.91	2.50	25.2	3.20	2.48	30.8	17.1	5.12	0	22.5	1.96	2.53	2.47	27.8	3.09				
30	28.1	1.60	2.23	23.5	2.21	1.89	29.9	12.4	5.42	0.13	22.5	1.92	2.48	2.00	23.7	2.96				
31	26.4	1.63	2.21	22.8	2.10	1.83	27.4	15.6	4.30	0	24.2	1.91	2.52	2.11	24.3	2.64				
32	26.5	1.64	2.28	21.3	1.64	1.50	26.9	7.2	4.52	0.12	21.0	1.93	2.44	1.11	18.8	2.11				
33	22.5	2.10	2.58	17.9	1.29	1.10	22.7	8.8	2.98	0.04	22.4	1.96	2.53	1.25	20.7	2.64				
34	26.2	1.86	2.53	20.8	2.20	2.05	31.9	10.1	5.11	0.26	22.1	1.93	2.48	1.97	21.9	2.66				
35	25.4	1.93	2.59	24.0	2.36	1.94	28.8	12.8	4.52	0.10	22.5	1.94	2.50	2.47	25.1	2.65				
36	21.9	1.42	1.82	58.3	6.46	5.46	77.6	24.7	20.59	0.04	25.6	1.72	2.31	3.67	46.6	10.95				
37	25.5	1.83	2.46	46.6	5.81	4.85	50.7	40.5	8.80	0	25.5	1.91	2.57	4.44	39.3	6.24				
38	21.0	1.98	2.51	34.4	5.61	4.67	39.7	31.3	8.33	0.42	19.2	2.01	2.49	4.45	29.6	6.23				
39	32.5	1.73	2.57	38.0	6.24	5.10	40.1	36.3	6.29	0.11	20.0	2.02	2.52	5.14	29.2	5.62				

samples of one soil, that were gently packed into small vessels perforated at the bottom, and placed in water. In these preliminary measurements the percentage of clay appeared to be inversely related to the apparent specific gravity and directly related to the pore space. Marchand (22), using the same method, did not confirm the former result with a series of Transvaal soils, but he did obtain a high correlation between clay and pore space.

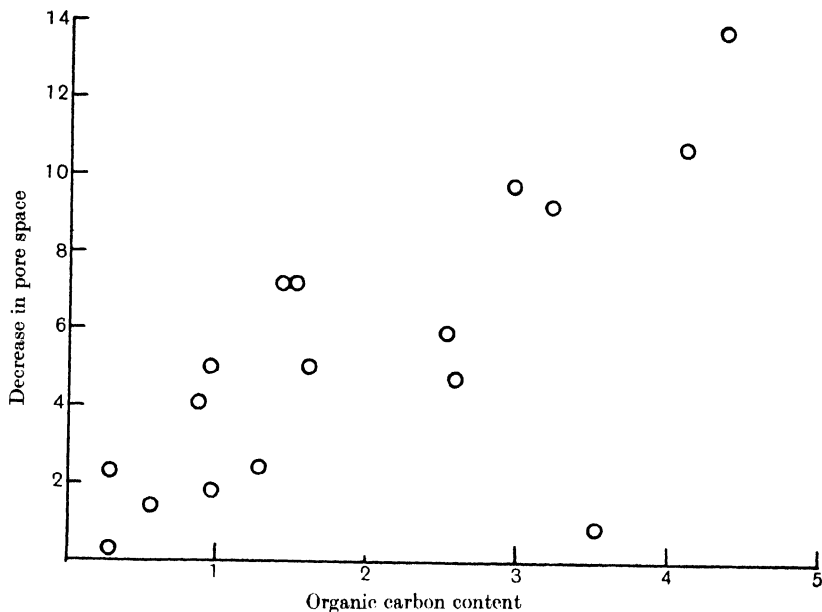


Fig. 1. Relation between organic carbon content and decrease in pore space after peroxide treatment.

In the present work there is no such relation between clay content and pore space in the dried plastic block, either for the original or the peroxide samples. It is evident that the thorough kneading tends to break down any compound particle structure and to encourage the individual grain to slip into closer packing. The bulk of the values for pore space fall around the average of roughly 26 per cent., although the clay and the organic matter contents vary widely. It is significant that the pore space of the peroxide treated soils is lower than that of the original samples in every case except two, and one of these (kaolin) is well within experimental error. This decrease may be attributed in a general way to the removal of the humified organic matter, permitting the individual mineral particles to slip into even closer packing than

before under the influence of the thorough kneading. The average reduction of pore space is 4-5 per cent. The reduction for the individual soil has been compared with the difference in the ignition values for the original and peroxide treated samples, which is a measure of the amount of humus removed by peroxide, but the correlation is hardly significant; the values for at least 10 of the soils fall widely away from the mean curve connecting the two variables. The figures for the organic carbon content of a certain number of the soils are available, and these show a close correlation with the decrease in pore space (Fig. 1), but the possibility that the complete series would show no better correlation than that of the ignition loss cannot be ignored. In any case the behaviour of the highly organic soils such as No. 36 (St Osyth Marsh top soil) requires further investigation: the pore space is increased from 21.9 to 25.6 per cent. after the action of peroxide.

(a) *General examination of data.*

The results of the complete examination of the associations between the different quantities can be expressed as correlation coefficients. From the scatter diagrams it appeared that the most important quantities were the percentage of clay (*C*), the moisture content at 50 per cent. relative humidity (*R*), the loss on ignition (*I*) and the sticky point (*S*).

Taking these in pairs, six correlations are possible, whose values are shown both for original and peroxide treated samples in Table III.

Table III. *Correlation coefficients between pairs of quantities.*

	A. Original soil				B. Peroxide treated soil		
	<i>I</i>	<i>R</i>	<i>S</i>		<i>I</i>	<i>R</i>	<i>S</i>
<i>C</i>	·364	·719	·317	<i>C</i>	·662	·760	·675
<i>I</i>	—	·388	·865	<i>I</i>	—	·386	·879
<i>R</i>	—	—	·503	<i>R</i>	—	—	·584

All these coefficients are definitely significant<sup>1</sup>; the lowest ( $r_{CS} = 0.317$ ) has a probability of more than 50 to 1. The table expresses the fact that in general the heavy clay soils have the highest ignition losses, moisture contents, and sticky points. It is significant that while four of the relationships are not appreciably altered by treatment of the soil with peroxide, the remaining two, connecting the clay content (*C*) with loss on ignition (*I*) and sticky point (*S*), are definitely increased. A closer

<sup>1</sup> The method followed is given in *Statistical Methods for Research Workers*, R. A. Fisher. (Oliver and Boyd, Edin. 15s.)

relation between  $C$  and  $I$  would certainly be expected, as with the removal of humified organic matter from the soil by peroxide the ignition loss will fall mainly on the clay fraction. The increased correlation between  $C$  and  $S$  indicates that the sticky point value is controlled both by the organic matter and some property related to the clay content. This point is returned to in more detail below (Section 5 (c)).

The highest correlations are between  $C$  and  $R$ , and between  $S$  and  $I$ ; this is especially the case with the original soils. Taking partial correlation coefficients the association can be found between  $R$  and  $C$  when  $I$  is eliminated and between  $S$  and  $I$  when  $C$  is eliminated. The results are:

$$r_{SI.C} = 0.843,$$

$$r_{RC.I} = 0.673.$$

On the other hand the association between  $R$  and  $I$  when  $C$  is eliminated and between  $S$  and  $C$  when  $I$  is eliminated are practically negligible:

$$r_{RI.C} = 0.194,$$

$$r_{SC.I} = 0.155.$$

These four results lead to the important conclusion that the value of the sticky point is largely controlled by the material in the soil that is driven off by ignition, while the moisture content in the soil at half saturation vapour pressure (a value that is close to the “air”-dry moisture content) is controlled much more by the clay content as determined in a mechanical analysis.

Further confirmation of this distinction is afforded by the negligible correlations between  $I$  and  $R$  when  $S$  is eliminated and between  $S$  and  $C$  when  $R$  is eliminated:

$$r_{RI.S} = 0.095,$$

$$r_{SC.R} = 0.015.$$

The association of the water contents represented by  $R$  and  $S$  mainly with the clay and ignitable material respectively, points to a difference of degree if not of kind in the manner in which the water is held in the two cases. In conformity with both direct and indirect measurements of vapour pressure of moist soils (13 a, 13 c), the moisture represented by  $R$  may be regarded as held in the minute interstices and capillaries between the clay particles, while the moisture content  $S$  is held by the colloidal material which includes the organic colloids of the humic material and the inorganic colloids of the clay fraction. It should be unnecessary to point out that there is no sharp line of separation between the two water contents, as quite apart from other considerations, this

would imply that only the two quantities—clay and loss on ignition—were influencing the results. As it happens, the correlation between  $S$  and  $R$  is still just significant when both  $C$  and  $I$  are eliminated:

$$r_{SR.IC} = 0.349.$$

However, the value of  $S$  obviously includes that part of the water held in the clay capillaries; if instead of  $S$  and  $R$  we obtain the correlation coefficient between  $(S - R)$  and  $R$ , with  $I$  and  $C$  eliminated as before, the value falls below the level of significance:

$$r_{(S-R)R.IC} = 0.140.$$

The high correlations between  $C$  and  $R$ , and  $I$  and  $S$ , therefore indicate a close causal association between the units of each pair of quantities.

The above conclusions are generally borne out by the partial correlations for the peroxide treated samples. These are given in Table IV, in which the values for the original soils are repeated for comparison.

Table IV. *Partial correlation coefficients.*

	Original soils	Peroxide treated soils
$r_{C,R}$	.843	.782
$r_{C,R,I}$	.673	.729
$r_{I,S,I}$	.155	.260
$r_{IR}$	.194	.303

Two other points of interest emerge from the comparison. In the original soils there is no apparent connection between  $I$  and  $R$  when  $C$  is eliminated, while in the peroxide treated sample the correlation is just significant (0.303). A possible explanation lies in the fact that in each case the ignition loss includes about the same amount of "constitutional water" from the clay, which in the case of the peroxide treated samples forms the main contribution to the total ignition loss. It is reasonable to suppose that the "constitutional water" is related to the colloidal properties of the clay and therefore to its minute capillary or reticulate structure, in which the equilibrium moisture content denoted by  $R$  is retained. Hence a correlation between  $I$  and  $R$  would be expected when the so-called constitutional water forms an appreciable fraction of the total ignition loss.

The second point to be noted is that the high correlation between  $C$  and  $S$  for the peroxide treated soils (Table III B) is reduced below the level of significance when the partial correlation coefficient between  $C$  and  $S$  eliminating  $I$  is obtained.

(b) *Relation between organic matter and single value determinations.*

The contribution of the organic matter in the soil to the various single values can now be discussed in more detail. In the first place the

relation between the difference in the ignition losses for the original and peroxide treated soils ( $I_o - I_p$ ) and the actual organic matter content of the soil must be considered. As already mentioned a certain number of determinations of total carbon by the wet combustion method are available. The results, corrected for carbonate carbon, are shown in Table V, together with the total organic matter calculated on the conventional basis by multiplying the corrected carbon figure by 100/55.

Table V. *Relation between carbon content and ignition losses.*

Soil	Difference in loss on ignition ( $I_o - I_p$ )	Total carbon content, % on air-dry soil	Carbon content excluding carbonate carbon	Total organic matter (carbon $\times$ 100/55)
1	3.2	2.59	2.51	4.56
2	4.78	4.37	4.37	7.95
3	5.15	3.53	3.53	6.41
4	2.08	1.61	1.61	2.92
5	1.46	0.88	0.88	1.60
6	2.27	1.43	1.43	2.60
7	0.60	0.56	0.56	1.02
9	2.99	2.81	2.59	4.71
11	2.48	1.51	1.51	2.75
12	3.88	4.14	4.10	7.45
14	1.35	2.95	2.95	5.36
15	1.53	0.95	0.95	1.73
16	-1.21	2.56	0.28	0.51
17	5.28	1.42	1.28	2.33
18	-0.52	0.54	0.29	0.53
19	1.78	1.41	0.96	1.75
20	5.53	3.20	3.20	5.81
36	9.74	8.64	8.64	15.70

It is evident, from Table V, that the difference in ignition values before and after peroxide treatment is in general less than the total organic matter, if the values for the latter can be assumed as approximately correct. The relation between total organic matter and ( $I_o - I_p$ ) is shown in Fig. 2, which suggests that about 75 per cent. of the total organic matter is represented by ( $I_o - I_p$ ), or, in other words, is removed by peroxide treatment of the soils. The value agrees with estimates of other workers and will be adopted in the present paper. As it is not possible to give organic carbon values for all the soils it is desirable to point out that the fairly close relationship shown in Fig. 2 may not hold for the whole series for the reason already mentioned at the beginning of this section (p. 748).

(c) *Physical significance of the sticky point value.*

As the sticky point ( $S$ ) is largely controlled by the ignitable material there should be a close correlation between the drop in its value as a result of peroxide treatment ( $S_o - S_p$ ) and the values of ( $I_o - I_p$ ). This

is so; the correlation coefficient between the two variables is 0.815. The sticky point is closely associated with the organic matter removed by peroxide and the ability to form an estimate of the actual amount so removed enables us to examine the relative contribution to the sticky point value of other factors besides organic matter. If we take the peroxide treated series we find, as already shown in Table III B, that the clay content  $C$ , and the sticky point  $S_p$ , are connected. The relationship is shown graphically in Fig. 3. Except for the four Craibstone soils

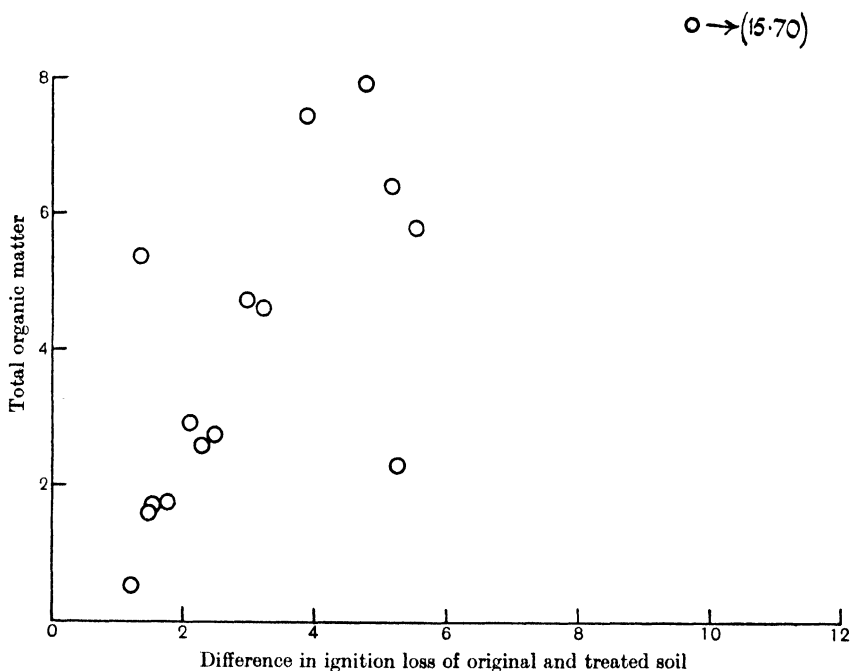


Fig. 2. Relation between total organic matter and difference in ignition loss of original and peroxide treated soils.

(Nos. 12, 13, 14, 15) and the highly organic marsh top soil (No. 36), whose sticky points are higher than the clay content would lead one to expect, the connection between the two variables is quite close, and can be represented quite fairly as linear. The line cuts the axis of  $S_p$  at about 16 per cent. moisture content.

This value represents the moisture content not associated with colloidal material, but present in the interstices of the plastic soil, *i.e.* the moisture content that would be held by a mass of sand worked up with water until its cohesion was about to disappear. The value of

16 per cent. is very close to 14.6 per cent. which is the calculated moisture content of an ideal soil in closest packing whose pore space is filled with water<sup>(23)</sup>. The pore space of the ideal soil in closest packing is 26 per cent. and we have already seen that the average pore space for the original

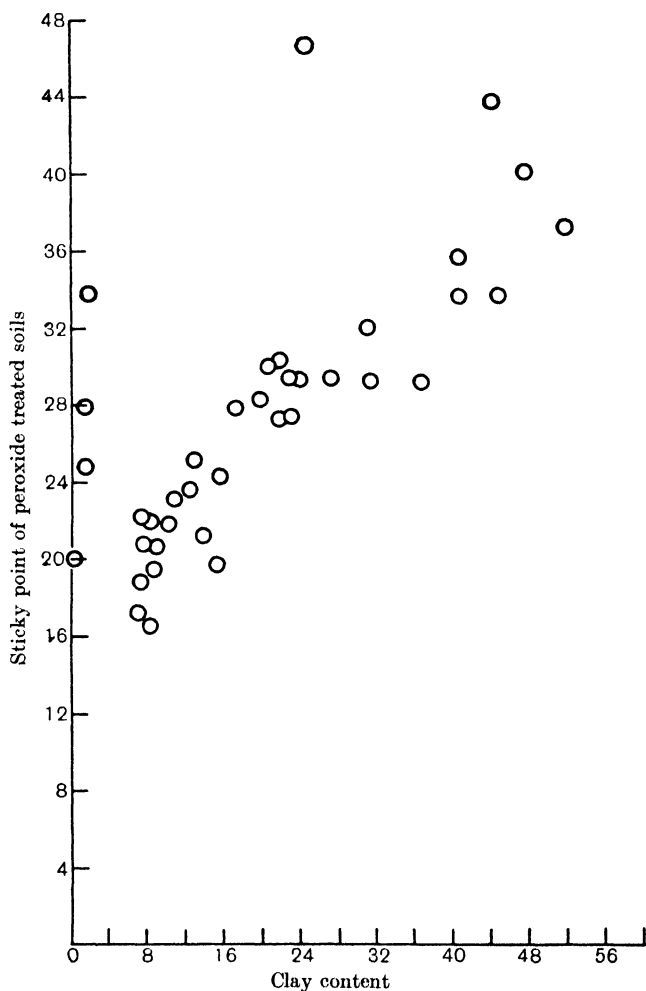


Fig. 3. Relation between clay content and sticky point of peroxide treated soils.

and peroxide treated samples is about 26 and 22 per cent. respectively. The process of kneading the soil in a sticky point determination produces a kind of statistical approximation to the condition in the ideal soil as far as the pore space is concerned, and hence we may with confidence identify the above mentioned moisture content of 16 per cent. as that

which occupies the pore space and is unassociated with colloidal material. This result is of interest in its bearing on Hardy's earlier work on West Indian soils (12). He developed the equation

$$\text{M.H.C.} = 23.5 + S,$$

where

M.H.C. = the moisture holding capacity,

23.5 = the film water,

$S$  = total colloiddally imbibed water,

which is identified with the sticky point determination on the assumption that, at the sticky point, the swollen colloidal material occupies all the space between the mineral grains. In a later paper (24) he recognised that the moisture content of the sticky point is not all associated with the colloidal material. On the basis of ideal soil calculations already described (23) the value of one-fifth, or 20 per cent. by weight, is taken as the interstitial moisture content for a coherent sand. The experimental values of  $S$  are therefore reduced by a figure equal to one-fifth of the percentage of sand present in the soil and the value  $(S - \% \text{ sand}/5)$  taken as a measure of the water held by the colloidal material; Hardy suggests that this value is an index of soil texture and of use in soil survey work. Wilsdon (25) gave reasons for assuming that the ratio of the colloiddally held water to the hygroscopic coefficient measured the vesicular coefficient of the soil colloids. Hardy's original and later postulates give respectively  $S/H$  and  $[S - (\% \text{ sand})/5]/H$  for this value, while the results given in the present paper lead to  $(S - 16)/H$ . In Table VI A and B these three ratios are compared for two series of experimental values given by Hardy.

The values for the modified ratios are naturally lower than those for  $S/H$ , since in each case the numerator of the fraction is reduced. Their values are also much steadier than those of  $S/H$ . Thus in Table VI A, values of  $S/H$  are higher for siliceous soils (top of table) than for laterites (lower half of table), as pointed out by Hardy, but this possible distinction is not borne out by the values of  $(S - 16)/H$ , which with three exceptions are singularly uniform. Similar results are shown in Table VI B.

Taking the whole data the value of  $(S - 16)/H$  lies, with rare exceptions, between 2 and 4. There are obvious limitations to the significance of this result, apart from the indefinite nature of the hygroscopic coefficient. Nevertheless, it is suggestive, and worthy of further attention. A variation in the numerical value of the vesicular coefficient would be expected in view of the known differences in composition of colloidal material from one soil to another. At the same time the variation in

$S/H$  seems larger than would be anticipated having regard to the smaller range of variation in other physical and physico-chemical properties over a wider range of soil types than those examined by Hardy, and hence the range of variations given by  $(S - 16)/H$  appears more probable.

Table VI. Comparison of values for vesicular coefficient.

A. Hardy's data (12)				B. Hardy's data (24)				
$H$	$S$	$S/H$	$(S - 16)/H$	$H$	$S$	$S/H$	$(S - 16)/H$	$S - (\% \text{ sand})/5$
2.0	17.7	8.8	0.85	3.6	28.1	7.8	3.4	4.8
4.4	25.2	5.7	2.1	9.0	47.8	5.3	3.5	4.8
5.1	26.4	5.1	2.0	4.7	35.3	7.5	4.1	5.9
6.8	33.3	4.9	2.5	7.3	42.4	5.8	3.8	5.2
7.1	35.4	4.9	2.7	9.6	44.7	4.7	3.0	4.4
8.5	37.2	4.4	2.5	10.3	46.5	4.5	3.0	4.2
7.2	33.7	4.7	2.5	11.0	53.8	4.9	3.4	4.6
8.1	37.5	4.6	2.7	12.7	51.5	4.1	2.8	3.8
11.5	45.1	3.9	2.5	4.0	25.0	6.2	2.3	3.2
11.9	42.0	3.5	2.2	3.6?	30.8	8.6	4.1	5.6
12.3	45.6	3.7	2.4	3.8	28.2	7.4	3.2	4.7
12.7	49.1	3.9	2.6	7.6	42.1	5.5	3.4	4.3
13.4	44.1	3.3	2.1	2.7	26.0	9.6	3.7	4.7
13.5	49.8	3.6	2.5	4.0	28.0	7.0	3.0	4.8
14.1	46.9	3.3	2.2	5.6	42.9	7.6	4.8	5.7
15.5	54.5	3.5	2.5	3.9	28.9	7.4	3.3	5.6
17.8	59.3	3.3	2.4	11.3	45.7	4.0	2.6	3.8
16.6	57.2	3.4	2.6	12.2	49.2	4.0	2.7	3.9
20.6	48.8	2.4	1.3	8.8	46.1	5.2	3.4	4.8
16.4	61.0	3.7	2.7	8.0	40.9	5.1	3.2	4.5
18.7	209.6	11.2	10.3	3.3	26.9	8.1	3.3	5.0
				11.6	49.6	4.3	2.9	4.0
				10.2	41.8	4.1	2.5	3.5
				8.9	40.1	4.5	2.7	4.0

If we assume that the value of the sticky point determination is the additive effect of (1) the 16 per cent. of water held in the interstices, (2) the water held by organic colloidal material, and (3) water held by inorganic colloidal material associated mainly with the clay fraction, it is possible to obtain a rough estimate of the relative contribution of items (2) and (3). The colloidal organic material is taken as that removed by peroxide treatment as the remainder is structural organic matter and probably without much direct effect on the sticky point; its value is therefore  $(I_o - I_p)$ . There is no obvious measure of the inorganic colloidal material; it might be regarded as proportional to the total amount of clay, to the clay surface, or to the loss on ignition of the mineral part of the soil. The last value has been adopted, and taken as the ignition loss of the peroxide treated soil less the organic matter still present. On the assumption that  $(I_o - I_p)$  represents three-fourths of the total organic matter, the amount still present after peroxide treatment is evidently  $\frac{1}{3}(I_o - I_p)$ .

The sticky points of the original and peroxide treated soils are respectively:

$$S_o = 16 + \alpha (I_o - I_p) + \beta (I_p - \frac{1}{3} (I_o - I_p)),$$

$$S_p = 16 + \beta (I_p - \frac{1}{3} (I_o - I_p)),$$

where  $\alpha$  and  $\beta$  represent the amounts of water associated with unit weight of organic and inorganic colloidal material respectively.

The mean value of  $\beta$  is obtained from the slope of the best straight line through the scatter diagram for the two variables in the second equation. Subtraction of the second equation from the first gives

$$(S_o - S_p) = \alpha (I_o - I_p),$$

from which  $\alpha$  is similarly obtained. In this case the points are rather widely scattered and the value of  $\alpha$  is approximate. The values of  $\alpha$  and  $\beta$  are respectively 3.3 and 2.8 so that the equation for the sticky point becomes

$$S_o = 16 + 3.3 (I_o - I_p) + 2.7 (I_p - \frac{1}{3} (I_o - I_p)).$$

The experimental values for  $S_o$  and those calculated from this equation are given in Table VII.

Table VII. *Comparison of calculated and experimental values for sticky point of untreated soils.*

Soil	$S$ calcd.	$S$ exptl.	$S$ exptl. - $S$ calcd.	Soil	$S$ calcd.	$S$ exptl.	$S$ exptl. - $S$ calcd.
1	35.1	34.3	- 1.8	21	(49.3)	40.6	(- 8.7)
2	47.0	56.7	9.7	22	35.8	29.8	- 6.0
3	41.4	46.0	4.6	23	27.1	21.4	- 5.7
4	26.2	27.3	1.1	24	35.4	37.2	1.8
5	24.3	19.7	- 4.6	25	32.9	37.0	4.1
6	24.1	22.3	- 2.8	26	49.5	33.1	- 16.4
7	39.3	26.8	- 12.5	27	56.9	30.8	- 26.1
8	34.1	30.1	- 4.0	28	30.8	26.8	- 4.0
9	41.3	28.8	- 12.5	29	31.0	39.8	- 0.2
10	32.4	29.3	- 3.1	30	30.0	29.9	- 0.1
11	24.7	27.4	2.7	31	27.3	27.4	0.1
12	39.6	44.7	5.1	32	27.7	26.9	- 0.8
13	37.8	43.1	5.3	33	24.2	22.7	- 1.5
14	40.1	36.9	- 3.2	34	29.2	31.9	2.7
15	25.4	22.2	- 3.2	35	28.0	28.8	0.8
16	(51.8)	35.0	(- 16.8)	36	69.7	77.6	7.9
17	45.5	38.8	- 6.7	37	39.5	50.7	11.2
18	(38.0)	39.1	(1.1)	38	38.4	39.7	1.3
19	41.6	33.7	- 7.9	39	33.3	40.1	6.8
20	41.3	45.4	4.1				

The agreement between experimental and calculated values is not good, and in fact only a qualitative agreement would be expected in view of the variety of assumptions made, and the experimental error in measuring the sticky points and the ignition losses. No attempt has been made, at this stage of the work, to improve the agreement by

altering the values of  $\alpha$  and  $\beta$ . Their values indicate that unit weight of organic colloid takes up  $(4/3) \times 3.3$ , *i.e.* 4.4 times its own weight of water, while the corresponding figure for the inorganic colloid is 2.7. These results show, therefore, that organic colloids take up slightly over 60 per cent. more water than an equal weight of inorganic colloids, when the latter are estimated in the manner suggested above. If, however, the clay content,  $C$ , be taken as a measure of the inorganic colloids in the above equations, the coefficient is found to be 0.5, which is only one-ninth of the coefficient for the organic colloids: a value in accordance with our knowledge of the relative efficiencies of the clay fraction and organic matter as water retaining colloids.

Table VIII. *Comparison of sticky point determinations by two workers A and B.*

Soil	Original samples			Peroxide treated samples		
	Worker A	Worker B	A - B	Worker A	Worker B	A - B
1	34.3	25.5	8.8	19.8	21.7	- 1.9
2	56.7	52.1	4.6	30.0	30.8	- 0.8
3	46.0	35.8	10.2	29.5	28.6	0.9
4	27.3	24.6	2.7	22.2	22.9	- 0.7
5	19.7	19.0	0.7	17.2	17.6	- 0.4
6	22.3	22.3	0.0	16.6	14.9	1.7
7	26.8	25.4	1.4	27.0	26.5	0.5
8	30.1	26.3	3.8	29.4	26.4	3.0
9	28.8	28.0	0.8	27.4	26.3	1.1
10	29.3	25.7	3.6	21.2	21.7	- 0.5
11	27.4	28.5	- 1.1	22.0	21.2	0.8
12	44.7	41.5	3.2	27.9	22.3	5.6
13	43.1	40.2	2.9	24.8	22.1	2.7
14	36.9	36.9	0.0	33.8	28.9	4.9
15	22.2	21.5	0.7	20.1	17.8	2.3
16	35.0	40.0	- 5.0	37.2	36.4	0.8
17	38.8	48.3	- 9.5	33.7	32.7	1.0
18	39.1	38.7	0.4	40.0	38.9	1.1
19	33.7	42.0	- 8.3	35.7	33.2	2.5
20	45.4	44.0	1.4	28.3	23.3	5.0
21	40.6	35.6	5.0	43.5	33.5	10.0
22	29.8	31.4	- 1.6	20.9	17.8	3.1
23	21.4	22.9	- 1.5	19.5	17.8	1.7
24	37.2	31.0	6.2	27.4	23.9	3.5
25	37.0	28.8	8.2	32.0	26.6	5.4
26	33.1	27.2	5.9	30.3	23.8	6.5
27	30.8	26.1	4.7	29.4	23.3	6.1
28	26.8	25.9	0.9	23.1	21.5	1.6
29	—	—	—	27.8	23.3	4.5
30	—	—	—	23.7	22.1	1.6
31	—	—	—	24.3	22.2	2.1
32	—	—	—	18.8	18.2	0.6
33	—	—	—	20.7	19.0	1.7
34	—	—	—	21.9	20.8	1.1
35	—	—	—	25.1	21.9	3.2
36	—	—	—	46.6	39.4	7.2
37	—	—	—	33.6	36.3	- 2.7
38	—	—	—	29.3	27.4	1.9
39	—	—	—	29.2	30.9	- 1.7

*(d) Accuracy of the sticky point determination.*

As already mentioned in Section 4, the recognition of the sticky point appears to present no difficulty after a little experience of the method has been gained. Nevertheless a certain amount of personal judgment is

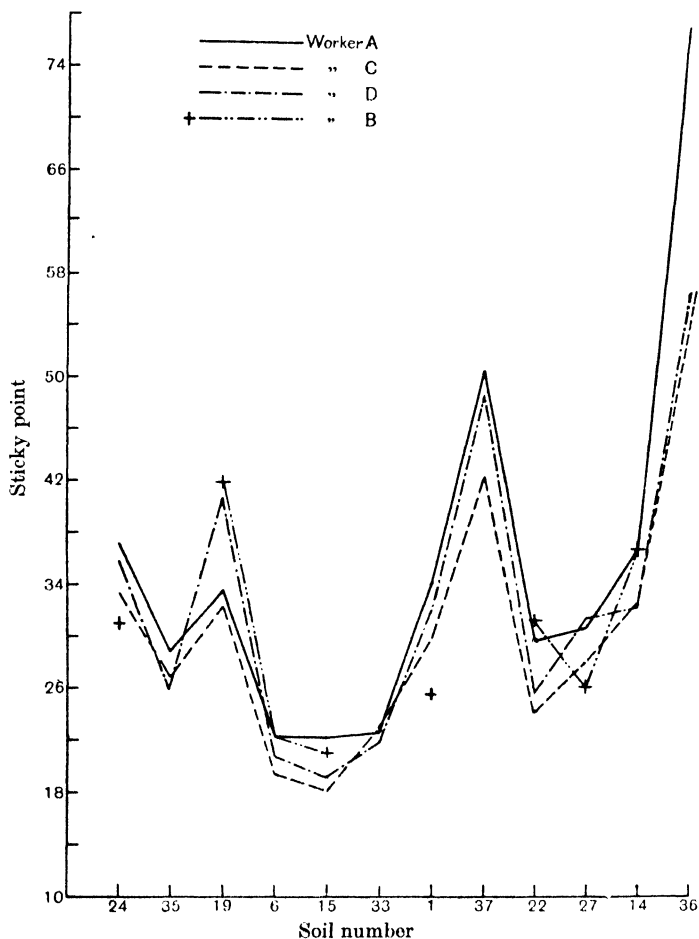


Fig. 4. Values obtained for sticky point of various soils by four workers.

involved and if the determination is to be generally adopted, some idea of the variation due to the personal equation is desirable. The sticky point determinations on the whole of the peroxide treated samples, and on 28 of the original samples were therefore independently repeated by another worker. The results are shown in Table VIII.

It is evident that worker *A* generally obtains a higher value, and with

a few exceptions, that the differences are small. Taking the 67 pairs of parallel determinations the differences are distributed as follows:

<i>A</i> ~ <i>B</i>	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11
No. of soils	16	17	7	7	4	6	3	1	3	1	2

Thus in 33 cases (one-half of the total) the divergence is less than 2 per cent. moisture content, while in 51 cases (three-quarters of the total) it is less than 5 per cent. Some of the larger discrepancies are probably due to experimental error, *e.g.* peroxide treated kaolin (soil No. 21). Others are on heavy clays of an exceptional nature (Nos. 17 and 19). It is suggestive that in friction measurements with heavy clays Haines<sup>(26)</sup> obtained two maxima, corresponding to (*a*) a clean cut with a knife through the plastic mass, and (*b*) a state of incipient “flooding,” in which the soil is just beginning to stick to external objects; it is possible that some similar effect explains the larger discrepancies here considered.

A further examination of the variation due to individual judgment was made with the help of two further workers, *C* and *D*. Twelve representative soils of the series were selected, and these two workers repeated the sticky point determinations. The experiment was made some considerable time after the work discussed in this paper had been concluded, and the workers *C* and *D* had only the written instructions for the sticky point determinations to guide them and had no previous experience with the method. Their results, and those of workers *A* and *B* for the same soils are set out diagrammatically in Fig. 4. It is evident from the figure that the trend of the results is the same in all cases, but, as with the more complete series of results for workers *A* and *B*, there are differences on individual soils. There is every reason to believe that the differences shown in Fig. 4 would be appreciably reduced after a little further experience<sup>1</sup>.

## 6. SCOPE OF SINGLE VALUE DETERMINATIONS.

The investigations discussed in this paper are not to be regarded as exhaustive, but rather as a preliminary study of the possibilities of simple single value determinations. The results are encouraging; the close association between the sticky point and colloidal material, and

<sup>1</sup> Later work, carried out by one of us (J. R. H. C.) on a number of South African soils will be discussed in a subsequent paper. It may be remarked here that replication of sticky-point measurements by two independent workers has been satisfactory—*e.g.* on 15 soils the average difference (neglecting signs) was 1.7 per cent., and there was no evidence that one worker obtained consistently higher values than the other.

between the moisture content at 50 per cent. relative humidity and the clay, strongly suggests that these two single value determinations are measuring soil properties that are reasonably distinct from one another. The determinations should therefore be correspondingly more useful, as coupled with a mechanical analysis, they enable soil to be specified from three different aspects. Again, repetition of the sticky point and ignition loss on peroxide treated soil gives additional information, offering the possibility—that needs further examination however—that the relative effects of organic and inorganic colloidal material can be distinguished.

There is urgent need for the adoption of additional methods of this nature. Under the impetus of the Russian school, soil classification and survey work have acquired a new and logical basis. The description of the soil profile however is still based very largely on a visual examination, and as long as this is so, a more detailed classification within any main soil type must remain qualitative, and dependent on the personal judgment of the observer. It appears that the single value measurements discussed in this paper are worth consideration as possible routine tests in connection with soil classification.

Apart from this, the methods have obvious application to the important physical and physico-chemical aspects of soil cultivation; this is now being followed up.

Further experience of the methods in the hands of different observers and on a wide range of soils is an essential preliminary to their general adoption. This has been arranged: the First Commission of the International Society of Soil Science decided at the Congress in Washington in June 1927 to organise an extensive series of co-operative experiments to this end, and the results will be reviewed at the next meeting of the Commission in June 1929.

## 7. SUMMARY AND CONCLUSIONS.

Numerous attempts have been made to devise an experimental method that, applied to a variety or a series of soils, enables them to be placed in an order closely reflecting their field behaviour or their most important physical characteristics. They are called "single-value" determinations as they endeavour to specify the soil by a single number, in distinction to the group of figures obtained, for example, from a mechanical analysis. A number of these methods are discussed in the present paper which contains an account of a detailed investigation on 39 soils of certain single value determinations.

The methods selected for study were chosen because (i) they required

only simple apparatus, and (ii) they appeared to be related to some distinct soil characteristic.

The list of measurements was as follows:

Percentage of clay.

Moisture content of soil in equilibrium with atmosphere of 50 per cent. relative humidity (the ordinary "air-dry moisture content" which was also determined, is close to this value).

Ignition loss of the dried soil.

Moisture content at the "sticky" point, which is defined as the point at which a thoroughly kneaded plastic mass of the soil is just about to stick to the fingers or to a knife.

The method of volume shrinkage developed by Haines was used to obtain the following additional quantities:

Moisture content of the saturated plastic block (this value closely approximates to the sticky point).

The pore space, true and apparent specific gravities of the oven-dried block.

The calcium carbonate present in each sample was determined, and on a number of the soils, total carbon content was also determined by the wet combustion method.

The most important feature of the present investigation was the repetition of the above measurements after the soils had been treated with hydrogen peroxide. Considerable experience of the effect of hydrogen peroxide on soil is now available owing to its inclusion in the Official British method of mechanical analysis, and our present knowledge indicates that it removes the humified and non-structural part of the organic matter without exercising more than a small solvent effect on the mineral portion of the soil. It has been assumed in the present investigation that the physical properties of the mineral portion are not appreciably altered by the peroxide. A comparison of the results for the original and peroxide treated soils thus gives an opportunity of comparing the relative contribution of the organic and the mineral portion of the soil to the single value measurements examined.

The main results obtained are set out below.

1. In spite of wide variations in clay and organic matter content the pore space of the kneaded blocks when oven dry fall closely around a mean figure of 26 per cent. This is reduced by 4-5 per cent. on the peroxide treated soils, and the effect is probably due to the ability of the grains to slip into closer packing under the influence of the thorough kneading, when the organic matter has been removed by the peroxide.

For the whole series of soils, however, the reduction in pore space is not related in a simple manner to the amount of organic matter removed. It is interesting to note that the pore space in the blocks of natural soils approximates to that of an ideal soil in closest packing (26 per cent.). Hence the process of kneading the soil in a sticky point determination produces a kind of statistical approximation to the ideal soil as far as total pore space is concerned.

2. Treatment with peroxide removes about 75 per cent. of the total organic matter present in the soil.

3. Correlation coefficients obtained for the various pairs of quantities examined express the general fact that the heavy clay soils have the highest ignition losses, moisture contents and sticky points.

4. An increased correlation between clay and sticky point for the peroxide treated soils suggests that the sticky point value is controlled both by the organic matter and some property related to the clay content.

5. When the associations are further examined by partial correlation coefficients, the sticky point is shown to be largely controlled by the colloidal organic and inorganic colloidal material, while the moisture content at 50 per cent. relative humidity is largely controlled by the actual clay content. There is independent evidence that this moisture is held in the minute interstices between the clay particles.

6. The sticky point approaches a lower limit of about 16 per cent. moisture content with very sandy soils containing little organic matter. This value is close to 14.6 per cent. which is the saturation moisture content of an ideal soil in closest packing, and it has already been shown that the pore space of this ideal soil and of the kneaded blocks of actual soil have approximately the same value. Hence the value of the sticky point moisture content is made up of (a) 16 per cent. of water held in the pore space, unassociated with colloidal material, and (b) water associated with inorganic and organic material. The division of the latter quantity into water associated with organic matter and inorganic clay colloids can be very approximately effected by assuming (i) that the difference between ignition losses of original and peroxide treated soil measures the effective organic matter, and (ii) that the ignition loss of the peroxide treated soil (less the organic matter still present) represents the clay colloid. On these assumptions it appears that the organic colloid takes up about 4.4 times its own weight of water, and the inorganic clay colloid 2.7 times its own weight. The approximate nature of the comparison must be emphasised, owing to the limitations in the assumptions

on which it is based. If the actual clay content be taken, instead of the ignition loss of the peroxide treated soil, as a measure of the inorganic colloid, the clay on a unit weight basis is only one-ninth as effective as the organic matter.

7. Measurements of the "vesicular coefficient" and "index of texture" made by Hardy are re-examined, allowance being made for the 16 per cent. of water not associated with colloidal material.

8. A comparison is made of the variation of sticky point determinations made by different workers and it is shown that satisfactory agreement can be secured after a little experience of the method.

9. The importance is stressed of introducing single value methods as an adjunct to the modern system of soil classification, and into soil physics.

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#### REFERENCES.

- (1) ROBINSON, G. W. (1922). *J. Agric. Sci.* **12**, 287-91.
- (2 a) Sub-committee of the A.E.A. (1926). *J. Agric. Sci.* **16**, 123-44. (*Rothamsted Memoirs*, **13**.)
- (2 b) Sub-committee of the A.E.A. (1926). *Agric. Progress*, **3**, 106-9.
- (2 c) Sub-committee of the A.E.A. (1928). *Agric. Progress*, **5**, 137-44.
- (3 a) *Proc. First Int. Congress Soil Sci. (Washington)*. (In the press.)
- (3 b) KEEN, B. A. (1928). *Soil Research*, **1**, 43-9.
- (4) ODÉN, S. (1915). *Int. Mitt. Bodenk.* **5**, 257-311.
- (5) COUTTS, J. R. H. and CROWTHER, E. M. (1925). *Trans. Faraday Soc.* **21**, 374-80. (*Rothamsted Memoirs*, **13**.)
- (6 a) KEEN, B. A. (1920). *J. Agric. Sci.* **10**, 44-71. (*Rothamsted Memoirs*, **10**.)
- (6 b) — (1922). *Trans. Faraday Soc.* **17**, 228-43.
- (6 c) — (1926). *Physics in Industry* (Inst. of Physics), **4**, 29-63. (*Rothamsted Memoirs*, **13**.)
- (7) FISHER, E. A. (1923). *J. Agric. Sci.* **13**, 121-43.
- (8) JOFFE, J. S. and MCLEAN, H. C. (1925). *Soil Sci.* **20**, 169-74.
- (9 a) ASHLEY, H. E. (1909). *U.S. Geol. Survey Bull.* 388.
- (9 b) GILE, P. L., et al. (1924). *U.S. Dept. Agric. Bull.* 1193.
- (9 c) WILKINSON, J. A. and HOFF, W. (1925). *J. Phys. Chem.* **29**, 808-15.
- (10 a) MUNTZ, A. and GAUDECHON, H. (1909). *Ann. Sci. Agron.* (3rd ser.), **4**, 393-443.
- (10 b) BOUYOUCOS, G. J. (1925). *Soil Sci.* **19**, 152-61.
- (11 a) TEMPANY, H. A. (1917). *J. Agric. Sci.* **8**, 312-30.
- (11 b) HARDY, F. (1923). *J. Agric. Sci.* **13**, 243-64.
- (11 c) HAINES, W. B. (1923). *J. Agric. Sci.* **13**, 296-310. (*Rothamsted Memoirs*, **12**.)

- (12) HARDY, F. (1923). *J. Agric. Sci.* **13**, 340-51.
- (13 a) PURI, A. N., CROWTHER, E. M. and KEEN, B. A. (1925). *J. Agric. Sci.* **15**, 68-88. (*Rothamsted Memoirs*, **12**.)
- (13 b) PURI, A. N. (1925). *J. Agric. Sci.* **15**, 272-83. (*Rothamsted Memoirs*, **12**.)
- (13 c) CROWTHER, E. M. and PURI, A. N. (1924). *Proc. Roy. Soc. A.* **106**, 232-42. (*Rothamsted Memoirs*, **12**.)
- (14) ALWAY, F. J. (1913). *Nebraska Expt. Sta. Res. Bull.* **3**.
- (15) SHULL, C. A. (1916). *Bot. Gaz.* **62**, 1-31.
- (16) CAMERON, F. K. and GALLAGHER, F. E. (1908). *U.S. Bureau of Soils Bull.* **50**.
- (17) SHAW, C. F. (1927). *Soil Sci.* **23**, 303-17.
- (18) KEEN, B. A., CROWTHER, E. M. and COUTTS, J. R. H. (1926). *J. Agric. Sci.* **16**, 105-22. (*Rothamsted Memoirs*, **13**.)
- (19) HAINES, W. B. (1925 and 1927). *J. Agric. Sci.* **15**, 529-35; **17**, 264-90. (*Rothamsted Memoirs*, **13**.)
- (20) FISHER, R. A. (1926 and 1928). *J. Agric. Sci.* **16**, 492-505. (*Rothamsted Memoirs*, **13**.) **18**, 406.
- (21) KEEN, B. A. and RACZKOWSKI, H. (1921). *J. Agric. Sci.* **11**, 441-49. (*Rothamsted Memoirs*, **11**.)
- (22) MARCHAND, B. DE C. (1926). *South African J. Sci.* **23**, 238-43.
- (23) KEEN, B. A. (1924). *J. Agric. Sci.* **14**, 170-7. (*Rothamsted Memoirs*, **12**.)
- (24) HARDY, F. (1928). *J. Agric. Sci.* **18**, 252-6.
- (25) WILSDON, B. H. (1921). *Mem. Dept. Agric. India, Chem. Series*, **6**, 154-84.
- (26) HAINES, W. B. (1925). *J. Agric. Sci.* **15**, 178-200. (*Rothamsted Memoirs*, **12**.)

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## SOME COMMENTS ON THE HYDROMETER METHOD FOR STUDYING SOILS

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Certain statements in the latest paper of Bouyoucos on his hydrometer method (1) call for an early reply from the writer. In this paper he deals specifically with the use of the hydrometer for determining (*a*) the colloidal content of soil and (*b*) the mechanical analysis of the soil, either in a few groups (sand, silt, and clay) or in a greater number of groups.

The author will not enter into the dispute on the measurement of colloidal content, this has already been taken up by Joseph (3), and recently Gile (2) has put forward some further pertinent considerations. However, the use of the hydrometer for mechanical analysis—or indeed its employment for any kind of observation—necessarily implies the satisfying of certain conditions before the method can be accepted as fulfilling what its sponsor claims for it. In a recent review of progress in soil physics (4) the author pointed out that, in essentials, Bouyoucos' hydrometer method was a determination of density of a soil suspension, and therefore corresponded to an isolated point on the summation percentage curve. The author stated, further, that "the determination was essentially qualitative since an appreciable density difference must exist in the suspension between top and bottom of the long hydrometer bulb after so short a period as 15 minutes." Bouyoucos (1) has now produced figures, and bases thereon the following remarks: "This statement is not justified. The method is quantitative with only a reasonable degree of inaccuracy." To these remarks the author can only reply, in turn, that—assuming such a self-evident fact requires any justification—the existence of an appreciable density gradient in the suspensions is well shown by Bouyoucos' own results. We have only to compare the values (1) given for the hydrometer readings of the top half and lower half of the suspension columns to see this. (See table on following page.)

As the hydrometer is calibrated in grams per liter the figures in the above table are not the actual densities of the suspensions, although these can be obtained by a simple transformation. It is more convenient here to deal with the former values. The results show differences in hydrometer readings between top and bottom halves of the same suspension of between 10 and 30 per cent, depending on the soil and the time of standing, and it must be remembered that in each case these values refer to the average of many different depths of

EXPERIMENT AND TIME OF STANDING	READING, UNDIS- TURBED COLUMN	DIFFERENCE IN READING, TOP AND BOTTOM HALF	DIFFERENCE IN READING, AS PER CENT OF UNDIS- TURBED COLUMN
	<i>gm. per liter</i>	<i>gm. per liter</i>	
1. Clay, 4 days.....	23.20	8.00	29.0
2. Clay, 2 hours.....	28.12	2.80	10.0
3. Clay, 15 minutes.....	36.17	3.43	9.5
4. Silt loam, 4 days.....	18.5	4.2	23
5. Silt loam, 2 hours.....	24.3	4.1	17
6. Silt loam, 15 minutes.....	29.4	3.9	13
7. Loam, 24 hours.....	10.5	3.0	27
8. Loam, 2 hours.....	12.2	1.4	11
9. Loam, 15 minutes.....	16.0	3.9	24

suspension. The actual density differences between the top and bottom layers of the whole column will be of course, very considerably greater than those corresponding to the above figures.

Bouyoucos also lays stress on the fact that the mean of the hydrometer readings for the upper and lower halves of the column is about equal to that of the undisturbed column, and considers that these results "refute the contentions or criticisms advanced by Joseph and Keen." It is evident, of course, that the average density of the suspension in the region where the hydrometer comes to rest in the original column of suspension will have a value intermediate between that of the superincumbent layers and that below the hydrometer, and in view of the averaging effect of the long hydrometer bulb employed it is not surprising that this intermediate value approximates to the mean. But the result has no other significance: it merely demonstrates the fact that a gradient of increasing density exists from top to bottom of the suspension. This density variation at different depths of the suspension is not constant, but progressively changes with time while the particles are slowly settling, and the actual manner in which it changes with time depends on the size distribution curve of the soil, and therefore varies from one soil to the next. Hence some convenient time has to be selected and laid down in the details of the method as the one to be used if comparable data are to be secured. The selection of 15 minutes, for example, is entirely arbitrary; this interval allows most of the coarser material to fall below the hydrometer range.

Hence in physical terms, the hydrometer technique consists in measuring at an arbitrary time the average density of a layer of suspension several centimeters in length, whose density is continually changing both with depth and time. The statement that the method is "essentially qualitative" is not a matter of personal opinion, but of fact.

Apart from this aspect it would appear that Bouyoucos has also not fully considered the inevitable disturbance produced in the mechanism of the

sedimentation by the presence of the hydrometer itself. Such disturbances are unfortunately inherent in all methods based on density or hydrostatic pressure measurements in a suspension. The reason for this was given in sufficient detail in a recent review (4) and need not be mentioned again.

It is evident that in spite of these facts the experimental results can, with due precaution, be repeated with very reasonable accuracy. (The results with the loam soil in lines 7-9 of the foregoing table, showing a minimum difference in density of top and bottom halves after 2 hours standing, in contrast to the regular increase with the other two soils, point at first sight to a rather considerable degree of inherent error; but this particular result may probably be fairly ascribed to some accidental disturbance). However, accurate repetition of a given result does not turn a qualitative method into a quantitative one, if we retain, as we ought, the strict meaning of these words.

In writing the foregoing, the author's only concern is that soil physics should have something of the precision of theory and experiment associated with the science of pure physics. Bouyoucos, in his legitimate enthusiasm over the experimental results of the hydrometer method, has implicitly claimed a fundamental basis for what, by its very nature, can only be an empirical method.

This is not intended in the least to deny its value as an empirical method. Bouyoucos has shown great skill in devising his apparatus and technique so that certain experimental errors are either nullified or mutually compensate one another, and in the present stage of development of soil physics the close relation of the results to those given by other empirical methods is a distinctly useful addition to our knowledge.

#### REFERENCES

- (1) BOUYOUCOS, E. J. 1927 The hydrometer method for studying soils. *Soil Sci.* 25: 365-369.
- (2) GILE, P. L. 1927 Colloidal soil material. *Soil Sci.* 25: 359-364.
- (3) JOSEPH, A. F. 1927 The determination of soil colloids. *Soil Sci.* 24: 271-274.
- (4) KEEN, B. A. 1927 First International Congress of Soil Science. First commission—soil physics and mechanics. *Soil Sci.* 25: 9-20.



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# PLASTOMETRIC STUDIES OF SOIL AND CLAY PASTES.

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(With Six Text-figures.)

## INTRODUCTION.

THE colloidal properties of the soil are now generally recognised as exercising a highly important contribution to its physical and physico-chemical characteristics. In the laboratory weak suspensions of the finer soil particles show a complicated range of colloidal properties, which are as yet incompletely understood. Nevertheless, the phenomenon of flocculation of such suspensions and the aggregation into compound particles characterising good tilth in field soils are two processes so strikingly analogous that the phenomena are considered to be closely related. Although attempts have been made to explain this relationship in exact terms it still resists solution. Evidently it is not sufficient to make direct comparison between such extreme conditions as flocculation in weak suspensions and the behaviour of soil in the field; a more comprehensive attack is needed in which the changing properties of a soil-water mixture are studied while the concentration of the soil is progressively diminished from that in the field to the weak suspensions (*circa* 1-2 per cent.) employed in studies of flocculation, cataphoresis, etc.

This investigation is now in progress in the Soil Physics Department at Rothamsted. It comprises a continuation of the field studies of cultivation processes and dynamometer measurements(1, 2), and laboratory work over the whole range of moisture content mentioned above. The latter may for convenience be divided into three ranges: the first in which the moisture content is of the same order as that in the field, the second in which the material is in the form of thick or semi-fluid pastes, and the third, or soil suspension range, in which the soil concentration rarely exceeds 5 per cent. by weight. In the first range, if the soil is well worked up with water a mass is obtained in which cohesive properties are paramount. The general behaviour over this stage is already known in its essentials(3). Over the intermediate range the paste displays both solid and fluid (or viscous) properties, while in the weak concentrations constituting the third range, this duality is shown by the

contrasting hydrophobe and hydrophyl properties of the colloidal system.

The present paper deals only with the intermediate range, and gives an account of some important relationships between the experimental results and other measurements made under very different conditions.

The experimental method adopted is to force pastes through capillaries of known dimensions under accurately measurable stresses and strain-conditions. As the range of viscous and solid properties displayed by materials under stress is of great importance in many industries, this experimental method has been much used, and the general descriptive term "plastometry" is applied to it. Much of the published work can be regarded as empirical as it is based either on defective experimental methods, or on an incomplete theory.

For this reason, and also because the plastometric study of soil has not hitherto received attention in soil studies, a brief statement will be given here of the theoretical basis together with a full description of the present experimental method and possible extensions of the technique.

#### THEORY OF THE METHOD.

The flow of *fluids* through capillary tubes is expressed by the classical equation of Poiseuille, which states that the quantity flowing in unit time,  $V$ , under an effective pressure,  $P$ , through a tube of length  $L$  and radius  $R$  is

$$V = \pi PR^4/8L\eta \quad \text{.....(1),}$$

the quantity  $\eta$  being the viscosity of the fluid.

In the case of liquids containing particles in suspension, and of pastes in which the relation between the two phases is of a more intimate character, the Poiseuille relationship no longer holds. Experimental work already published from this laboratory (4) and further results (5) show that the flow of a soil or clay paste through capillary tubes systematically changes its character as the pressure applied is increased. Considering first the case of a single capillary tube, four stages can be distinguished:

*Stage 1.* The paste does not begin to flow until a certain pressure has been reached. Below this critical pressure therefore the mixture is behaving as a solid.

*Stage 2.* The paste moves as a rigid cylindrical plug through a thin envelope of the fluid, which adheres both to the wall of the tube and to the plug. Over this range the volume of flow in unit time increases linearly with the pressure applied.

*Stage 3.* The diameter of the plug decreases. In the annular region surrounding the plug, the paste has a stream-line flow. The general motion can be pictured as similar to the drawing out of a telescope, the eyepiece tube representing the central plug and the remaining tubes the stream-line shells surrounding it. The volume of flow therefore increases at a faster rate than the pressure applied. As the pressure is increased further the diameter of the central plug progressively decreases. Over this region the bulk of the paste has a stream-line motion, with the result that the relation between volume of flow and applied pressure rapidly approaches linearity.

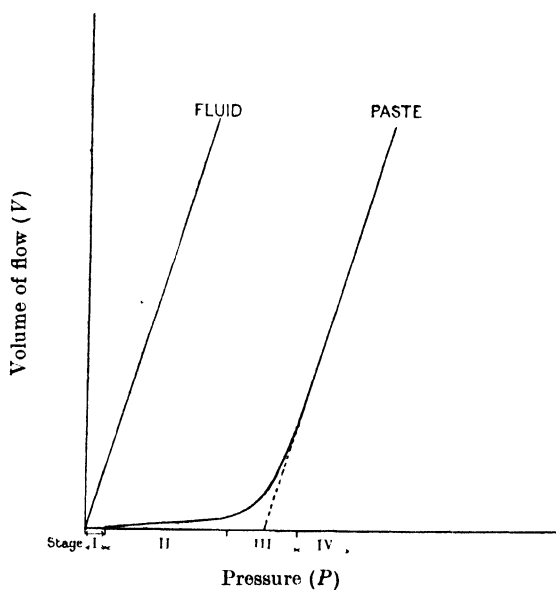


Fig. 1.

*Stage 4.* Over this region the flow is so nearly stream-line throughout that the error involved in assuming linearity between volume of flow and pressure becomes negligible.

Hence when the volume of flow is plotted against pressure applied, a curve of the type shown diagrammatically in Fig. 1 is obtained. Actually a complete experimental curve shows such a wide range of rates of flow that, on a vertical scale suitable for Stages 3 and 4, the earlier stages would be hardly appreciable. The flow in the later stages is about one hundred times as fast as at the beginning.

Evidently the behaviour of the paste in general differs strikingly from that of a true liquid, for equation (1) shows that for a given capillary

the relation between the volume flow  $V$  and pressure  $P$  for a fluid is represented (as in Fig. 1) by a straight line passing through the origin. But as Stage 4 in the curve for paste is sensibly straight and constitutes the greater part of a complete experimental curve, there is evidently some similarity between the behaviour of a paste over this range, and that of a true liquid.

For the purpose of examining this behaviour more conveniently it is usual to plot the results in a slightly different manner from that used in Fig. 1. The equation for a fluid (equation 1) can be written:

$$V/\pi R^3 = (PR/2L) (1/4\eta) \quad \dots\dots(2).$$

Hence if a number of different sized capillary tubes were used in turn, and a series of measurements of  $V$  for different values of  $P$  were made on each tube, the curve obtained on plotting  $V/\pi R^3$  as ordinates, and  $PR/2L$  as abscissae, would be a single straight line passing through the origin as shown in Fig. 2.

The slope of the curve is evidently  $1/4\eta$ , *i.e.* a numerical constant  $(1/4)$  multiplied by the inverse of the viscosity  $\eta$ . The inverse viscosity is usually called the fluidity. If the results had been plotted on the basis of Fig. 1, a family of straight lines would have been obtained, the slope of each depending both on the fluidity of the liquid and on the dimensions of the capillary. The method adopted in Fig. 2, however, is not only

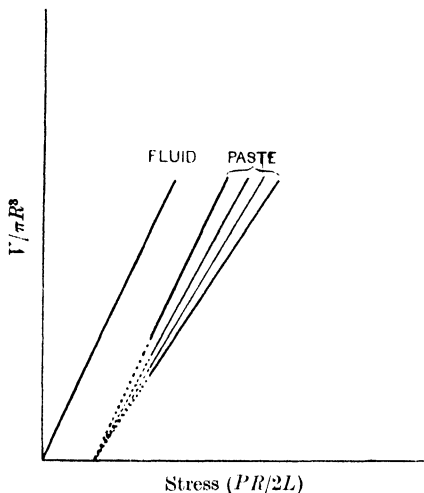


Fig. 2.

more convenient graphically, but has a definite physical significance. For the pressure  $P$  is, by definition, a force per unit area; the total force applied across the face of the capillary of radius  $R$  is thus  $\pi R^2 P$ . In the steady state of flow this pressure is balanced by an equal and opposite force, namely the total stress round the walls of the capillary, whose area is  $2\pi RL$ . Hence the stress per unit area of the wall is  $\pi R^2 P / 2\pi RL$ , or  $PR/2L$ . Thus the abscissae in Fig. 2 represent stresses per unit area irrespective of the capillary dimensions. Further, the slope of the curve for a true fluid represents one-fourth of the fluidity. When the same

method of plotting is applied to the results for a *paste* (attention being confined to Stage 4) it is found as a general rule that a single straight line is not obtained. Instead a family of straight lines is given, one for each capillary used. It is found as an experimental fact, for all clay and soil pastes so far investigated, that when the straight line portion (Stage 4) of each curve is extrapolated to the stress axis, they all meet at a point. A series of such curves is shown diagrammatically in Fig. 2. Two deductions can be made: first, that there is a certain definite shearing stress for the material represented by the common intercept of the extrapolated curves on the stress axis, which, if subtracted from the total applied stress, then enables the graph *for any one capillary* to be treated as analogous to the single graph that completely represents the behaviour of a true fluid (geometrically this is equivalent to shifting the origin along the axis of stress to the point of intersection); secondly, the fact that a family of curves is obtained with a paste indicates that the fluidity measurement (the slope of the curve) is not independent of the dimensions of the capillary. The theory accounting for this departure and the method of treating the results to obtain a measure of the fluidity is dealt with later.

This definite shearing stress is usually called the "static rigidity" of the system. It represents the energy required just to cause the paste to flow, and is a measure of the solid cohesive properties of the system. Objection has been raised to this conception on the grounds that the intersection with the stress axis, being obtained by extrapolation of an asymptotic portion of the curve, lies outside that curve, and has therefore no physical significance<sup>(6)</sup>. On the other hand, it appears valid to assume that over Stage 4 of the curve the energy represented by the "static rigidity" is used up in overcoming the friction between solid particles of the paste, and the effective stress causing flow is therefore less than the total stress by a constant amount.

No equation has yet been formulated that completely defines the relationships between volume of flow per second and applied pressure outlined above. Buckingham<sup>(7)</sup> put forward one consisting essentially of two terms, one of which covers Stage 2 (solid plug flow) and the other Stages 3 and 4 (partial to complete stream-line flow). The fact that in general a series of curves is obtained for different capillaries when  $V/\pi R^3$  is plotted against  $PR/2L$  (see Fig. 2) shows that Buckingham's equation does not directly apply to soil and clay pastes. But so far as flow as a solid plug is concerned, earlier work in this laboratory<sup>(1)</sup> has shown that the following equation (which is a

slightly modified form of Buckingham's first term to include Stage 1) is satisfactory:

$$V = \frac{\pi R^3 \epsilon \phi (P - a)}{2L} \dots\dots(3),$$

where  $\epsilon$  is thickness of water envelope through which the plug is flowing,  $\phi$  is its fluidity (not necessarily that of water in bulk),  $a$  is the pressure attained when the plug just begins to move and  $V$ ,  $R$ ,  $P$  and  $L$  have the meanings already given. But even this equation does not hold invariably; if, instead of using a paste made from moist soil fresh from the field, the soil is air-dried before use, the simple plug motion of Stage 2 does not generally occur, and this part of the volume-pressure curve is no longer a straight line.

This effect is almost certainly associated with the colloidal properties of the soil, but the exact mechanism of the process is not yet understood. The change is certainly a reversible one, because pastes made from moist surface soil do not show this abnormal behaviour, although the soil frequently passes through the air-dry condition in the field. Nevertheless, the process can hardly be a *simple* reversible one, because there is a wide variation in the ease with which air-dried samples of soil recover the property of giving a simple plug flow of the type defined by equation (3). The phenomenon requires further elucidation. Inspection of equation (3) shows that the properties defined by the constants  $\epsilon$ ,  $\phi$ , and  $a$ , may be affected by the air-drying, since the other symbols refer only to the dimensions of the capillary and to the recorded experimental measurements. Experimental work has shown that the values of  $a$  and of the product  $\epsilon\phi$  are the same in a "recovered" soil and in the original soil before air-drying.

With regard to the third stage of plastic flow, no satisfactory equation is yet available. Reiner<sup>(8)</sup> and later Buckingham<sup>(7)</sup> independently derived equations based on the conception that the plug type of flow was gradually replaced by stream-line flow. Although this conception is known to be qualitatively correct the data obtained in this laboratory do not conform completely to the equations of Reiner and Buckingham.

In the fourth or stream-line stage, as already pointed out, the divergence of the curves connecting  $V/\pi R^3$  and  $PR/2L$  for different capillaries (see Fig. 2) precludes the possibility of directly obtaining a simple viscous constant independent of capillary dimensions. Considerable attention has been devoted to this point, and a full account is given elsewhere<sup>(5)</sup>; in the present paper only a brief outline will be given. It has already been shown (see Fig. 2 and discussion thereon) that over

the fourth region of plastic flow it is necessary in any case to modify the Poiseuille equation by subtracting from the pressure ( $P$ ) an amount equivalent to the static rigidity, so that equation (1) becomes

$$V = \frac{\pi R^4 (P - c)}{8L\eta'} \quad \dots\dots(4).$$

The quantity  $\eta'$  corresponds to the viscosity  $\eta$  of a true fluid, and is called the "pseudo-viscosity."

If this modified equation be applicable to the fourth stage of flow, then on plotting  $V/\pi R^3$  against  $PR/2L$ , as in Fig. 2, the points should all be on one straight line irrespective of the radii of the capillary tubes used, while actually it is found that a separate straight line is given for each capillary. To account for this divergence it is assumed that the properties of the paste become modified in the immediate neighbourhood of the tube wall, so that the rest of the paste has a different mean velocity from that to be expected from the equation (4). The experimentally measured total volume of flow ( $V$ ) is therefore made up of two quantities, one of which obeys equation (4) while the other does not; the hypothesis given above enables the experimental results to be interpreted by eliminating the latter. Equation (4) can be written

$$\frac{V/\pi R^2}{(P - c) R/2L} = \frac{R}{4\eta'} \quad \dots\dots(5).$$

The left-hand side of this equation is evidently the mean velocity of flow for the whole paste ( $V/\pi R^2$ ), over the effective stress  $(P - c) R/2L$  at the wall of the tube. We can now modify this equation by subtracting the quantity  $\sigma_0$ , which represents the contribution of the modified layer at the wall:

$$\frac{V/\pi R^2}{(P - c) R/2L} - \sigma_0 = R/4\eta' \quad \dots\dots(6).$$

To apply this equation, the slope of velocity-stress curve

$$\left[ \frac{V/\pi R^2}{(P - c) R/2L} \right]$$

for each tube is plotted against the radius of that tube. It is found that the points for the different capillaries fall on a straight line, which on extrapolation gives intercept  $\sigma_0$  on the slope axis. This intercept is a measure of the extent of the anomalous behaviour near the wall, while the slope of the curve, as is also evident from equation (6), is equal to  $1/4\eta'$ , where  $\eta'$  is the pseudo-viscosity of the bulk of the system.

This equation is found to hold very well over a wide range of concentration and materials, subject to the restrictions that the capillary

diameter must not be so small that the modified layer forms an appreciable portion of the total flow, nor so large that stream-line motion is replaced by turbulent flow.

It is clear that the actual values of the plastic constants dealt with in the above discussion will depend on the concentration of paste used. In comparing different soils it is necessary to work either at the same concentration of paste, or to use each material at a number of different concentrations.

It will be realised from the above account of the theory of flow of plastic pastes, that although it is now possible to evaluate several general constants representing the plastic behaviour, the subject is a complicated one, and there is still much work to be done on the dynamics of the flow.

#### EXPERIMENTAL METHODS.

*Arrangement of apparatus.* The plastometer bulbs were made from two pipettes by bending round and cutting off the ends, the surfaces being ground flat (see Fig. 3). The volume of the bulbs used depends on

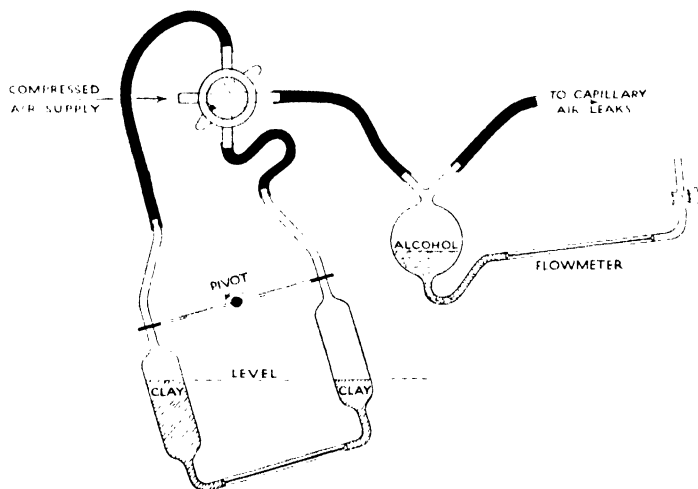


Fig. 3. Diagram of plastometer.

the range of stress to be investigated. Convenient sizes are 10, 50 and 100 c.c. each. The capillary tube is connected between them by means of short lengths of rubber tubing, the ends of the capillaries being ground to fit against the ends of the bulbs. The whole system is mounted on a stand which can be rotated on a pivot, so that when the paste is forced alternately from one bulb to the other, the surface of the paste in the bulbs

can be kept roughly in one and the same horizontal plane. This is effected by a chain which is fastened to the two ends of the stand, and passes over a toothed wheel above the apparatus, connected by means of a pulley system to a level-control wheel turned by hand.

The top ends of the bulbs, which are bent towards each other slightly for convenience, are connected by means of flexible rubber tubing to a four-way tap so that each can be connected alternatively to the air-pressure supply and the flowmeter. In the first shearing, one bulb is connected to the air-pressure supply, and the other to the flowmeter; in the second shearing the connections are reversed, and so on.

Compressed air is supplied from a foot pump and is stored at a pressure marked by a rough manometer, in a large stone bottle as a reservoir. It is released from this through a sensitive valve into a second reservoir (in order to insure a perfectly steady application of pressure) which is connected to the plastometer bulb. The second reservoir also connects with (1) a mercury manometer to measure higher pressures, (2) a water manometer for use at low pressure, (3) an exhaust to release pressure suddenly, and (4) a capillary leak to lower pressure slowly and steadily if required.

The paste can then be forced through the capillary at a series of different pressures which can be applied steadily, and measured accurately.

In order to measure the volume of flow per second, the air displaced by the flowing mass of paste is caused to pass through a flowmeter as shown in the figure, before escaping through one of a series of long capillary tubes. The flowmeter consists of a sensitive alcohol manometer set at a small angle to measure the pressure at the entrance to the air capillary. Since air is a true fluid, this pressure is proportional to the volume of air passing through the air-leak capillary per second, and hence to the flow per second of the paste in the plastometer.

It is necessary to adjust the zero of the flowmeter manometer before each run. When this has been done, the paste is forced through at a series of different pressures, and for each, the reading on the flowmeter is recorded. To convert this into c.c./sec. a factor is used depending on the dimensions of the particular capillary air-leak in use. To determine the factors, a glycerine-water mixture of convenient viscosity is passed through the apparatus, and the time required for it to flow through a bulb of known volume is recorded on a stop watch. This is repeated at different readings on the flowmeter, the reading being held constant during each run by keeping the level suitably adjusted.

In this way, a calibration curve for the flowmeter is obtained for each capillary air-leak.

The capillary and bulbs, the flowmeter, and the capillary air-leak are all kept in a thermostat at 25° C.

*Plastometer capillaries.* These are cut from the most uniform capillary tubes obtainable, and are carefully ground at the ends. The length is measured directly, and the radius is found by weighing the amount of mercury which will just fill the capillaries at a known temperature. This has been found more accurate than a determination by a viscometric method. Capillaries of very widely different lengths and radii have been used, but for ordinary purposes about 12 cm. is found a convenient length, with a radius of from 0.04 to about 0.12 cm.

*Preparation of materials.* It has not been found possible to get satisfactory results with any paste containing solid particles which will not pass through a sieve with 100 mesh per inch. Soils, whether direct from the field, or air-dried, are made up into a paste with distilled water and forced through such a sieve, the coarse sand remaining on the sieve being discarded.

For this reason the plastometer is not suited to the study of soil containing a high percentage of coarse sand. It is also necessary to pass pastes prepared from clay fractions through sieves, since the presence of a single particle of grit would vitiate the experiment.

The moisture content of the sieved paste is then determined by heating a sample in an oven at 160° C. for one hour. (This gives results closely correlable with those obtained in a steam oven for 24 hours.) In preparing a series of pastes from soils direct from the field at the same concentration in the paste, it is found unsatisfactory to determine the moisture contents of the original soils and then to add the required amounts of water to form the pastes, since soils do not take up the water evenly. The much more laborious process of drying down the soils gently in the laboratory until each attains a given moisture content and then adding the same quantity of water to each is best employed.

The paste is brought up to temperature by immersion in a bottle in the thermostat, and then sucked into the bulbs, and the apparatus is connected up, a capillary of suitable dimensions being used. It is best to get curves for each sample with at least 4 or 5 capillary tubes of different dimensions. The paste should be transferred to a bottle and shaken between runs with the different capillaries. Before any readings are taken, the paste is always sheared once through the capillary. This not only enables the operator to decide on the best series of pressures

to apply, but removes any irregularities still present in the paste, and eliminates the phenomenon known as thixotropic structure.

The flowmeter readings for a series of constant pressures are then recorded, the number of separate applications of pressure during each shear depending on the rate of shear used, and the dimensions of the bulbs. For accurate work, the bulbs are finally connected to each other without a capillary and a curve is obtained giving the amount of pressure taken up by the bulbs themselves for any given volume of flow per sec. This is subtracted from the observed pressure before calculating the stresses.

Since the range of the flowmeter for a given air-leak is small, it is best, if a wide range of stresses is to be investigated, to use at least two different air-leaks during each run.

Although this process sounds somewhat laborious it has been found possible to take the measurements quite quickly. Thus, to determine the plastometric constants of a given paste at a single concentration even with five separate capillaries would involve only about  $1\frac{1}{2}$  hours experimentation and an hour's calculation. If the operator is sure that he is working on the straight-line portion of the curves, much time can be saved by applying only three different pressures for each capillary and allowing the whole of the paste to flow across for each reading—taking a mean flowmeter reading for the whole run. In this way quite satisfactory constants have been obtained for ordinary purposes necessitating only three calculations of stress and mean velocity for each tube.

The above apparatus and technique, although considerably modified for soil work, are essentially those of Bingham<sup>(9)</sup>, who has applied a similar method to the study of paints, etc.

#### EXPERIMENTAL RESULTS.

Work is still in progress on the relation between the pseudo-viscosity of soil and clay pastes and other physical properties of the soil, and will form the subject of a later paper. The present account will be confined to certain relations found to exist between the static rigidity and various field characteristics of soil. The full meaning of these results have still to be worked out; their immediate importance lies in the demonstration that the constants determined by plastometric measurements in the laboratory are related to field and laboratory measurements made under very different experimental conditions.

*Static rigidity and dynamometer measurements.* Numerous measurements with a dynamometer of the resistance offered by soils to the

passage of cultivation implements have been made at Rothamsted (1, 2) and the results have been shown to represent a characteristic property of the soil. Considerable variations in the resistance may be found from point to point over a given level area, and these variations bear no obvious relationships to such factors as moisture content, percentage of clay, etc. Nevertheless, the close concordance between successive results even over a series of years, unquestionably shows that the dynamometer measurement represents some definite soil property. It can conveniently be regarded as an integrated measure of the various physical properties such as cohesion and plasticity (using these terms in their general meaning) that are brought into play when the soil mass is cut by the coulter and share and turned over by the mouldboard.

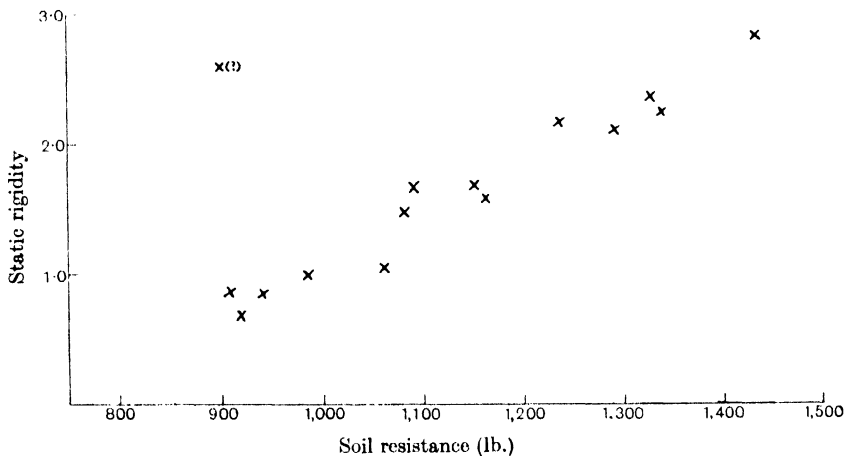


Fig. 4. Static rigidity and soil resistance, Broadbalk field.

Samples of soil were taken from definite parts of various fields for which full dynamometer records exist, and made into pastes of the same concentration as described above.

The relation between the static rigidity of the paste and the dynamometer measurement for that part of the field from which the particular sample was taken is shown for Broadbalk field in Fig. 4. It is evident that a close correlation exists. The result of a similar experiment for Sawyers field are given in Table I. The correlation coefficient is again significant, but the results are not so striking as those for Broadbalk. Table I also shows the values for the light sandy soil at Woburn, and here the relation is not so significant. This may indicate that it does not hold for sandy soils, although it should be remembered that the coarse

sand is removed before plastometric measurements can be made, and as the Woburn soil contains some 50 per cent. coarse sand it is perhaps not surprising that the results show so little regularity.

Table I. *Dynamometer measurements and static rigidity.*

1. Sawyers field, Rothamsted, air-dry soil			2. Stackyard field (permanent barley plots), Woburn, air-dry soil		
Plots	Dynamometer measurements	Static rigidity	Plot	Dynamometer measurements	Static rigidity
H 1	1220	1.98	5 BX	214	1.46
A 1	1235	1.96	2 AA	225	2.00
H 2	1244	2.12	8 BB	230	1.94
F 1	1280	2.14	9 B	235	2.40
B 3	1280	2.16	8 A	238	1.18
G 3	1306	2.18	10 A	253	2.80
G 4	1350	2.51	2 BB	255	3.33
C 1	1350	2.14	10 B (top)	287	1.50
H 4	1360	2.44	11 A	305	2.47
C 5	1410 (?)	2.07			
E 4	1520	2.77			

There is one point on the Broadbalk curve which falls very far from the general line. This refers to the sample taken from the plot which has received heavy annual dressings of dung for the past 80 years. The pseudo-viscosity was very high, and an exact determination of static rigidity was difficult; nevertheless, it may well constitute a real exception to the relationship which, as is shown also in Table I, is not necessarily of general application.

*The effect of chalk, lime and cyanamide.* Dynamometer measurements, made several years after the application of heavy dressings of chalk, have shown that the soil resistance is appreciably reduced as compared with untreated soil. Experiments with smaller dressings of chalk and equivalent dressings of lime have shown no appreciable reduction in soil resistance, at any rate in the year following the application. It is of interest to see whether there is any effect on the static rigidity. A large sample of an acid surface soil adjacent to the Park grass plots, Rothamsted, was divided into a number of separate portions, to each of which was added a certain quantity of chalk, lime, or cyanamide, with thorough admixture. The amounts of lime, chalk, and cyanamide added to the soil were calculated on the basis that one part per thousand corresponded to 1 ton per acre in the field. After definite periods of time, a sample of each of these was removed, and made up into a paste at a moisture content of 50 per cent. in the usual way. The paste stood for 1 hour during the moisture content determination, and then, after the slight

necessary adjustment in concentration, was tested in the plastometer. The static rigidities are given in Table II. This table shows that:

- (1) Large quantities of chalk reduce the static rigidity but take some days to effect the reduction.
- (2) Large quantities of slaked lime likewise reduce the rigidity, but the effect is much more rapid.
- (3) Quantities of chalk and lime too small to affect the dynamometer measurements appreciably reduce the static rigidity.

Table II. *Effect of chalk, lime and cyanamide in various quantities on static rigidity of 50 per cent. paste, Park grass surface soil.*

Sample	Treatment	Duration (days)	Static rigidity (C)
K	Control	—	1.43
L	50 tons chalk p.a.	0	1.43
		7	1.18
		25	0.65
M	38 tons slaked lime p.a.	0	0.47
		7	0.15
		25	0.19
N	10 tons slaked lime p.a.	0	1.12
		11	0.77*
		26	0.86
O	2 tons cyanamide p.a.	0	1.41
		10	0.80
		24	0.56
P	1 ton slaked lime p.a.	0	1.39
		9	0.86
		26	0.94*
Q	$\frac{1}{2}$ ton cyanamide p.a.	1	1.30
		27	0.55

\* Results known to be somewhat uncertain.

*Static rigidity and concentration of paste.* The importance of the concentration of soil in the paste was referred to at the end of the section dealing with the theory of the method, for it is clear that this factor must be taken into account in all measurements. A series of experiments was made therefore on the relation between concentration of paste and static rigidity, for a wide range of soils and clays. The results are shown in Fig. 5. The relation is evidently complex but the relative position of the curves on the diagram gives a clear indication of the extent to which colloidal properties are shown by the various materials. The curves for bentonite and the clay fractions are at the left-hand end; in other words, these materials display static rigidity at low concentration, whereas the lighter soils (at the right-hand end of the diagram) must be used in much higher concentrations before measurable static rigidity occurs.

*Static rigidity and "sticky point."* Recent work by Keen and Coutts on a wide variety of soils(10), and by Coutts(11) on Natal soils, has shown that the moisture content at which a kneaded and plastic mass of soil is about to become sticky is a well-defined quantity that promises to be of service as a "single-value" soil measurement. Although there are exceptions, in general the sticky point appears to give a fair measure of the extent to which colloidal properties of the soil are developed. The

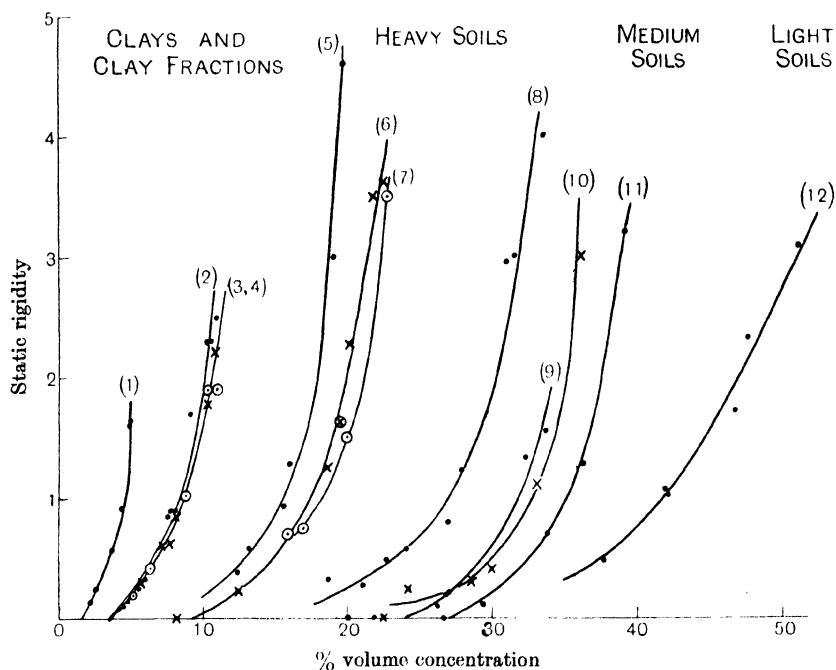


Fig. 5. Variation of static rigidity in concentration of material. (1) Natural bentonite. (2) Clay fraction, Broadbalk field. (3) Oolitic clay. (4) Liassic clay. (5) Fiji lateritic soil. (6) Natural kaolin. (7) Gold Coast soil. (8) Surface soil from parkgrass field (Rothamsted). (9) Broadbalk soil, farmyard manure plot. (10) Broadbalk soil, unmanured plot. (11) Beaumont alluvial subsoil. (12) Punjab soil.

results given in the preceding paragraph also suggest that the static rigidity-concentration curve may show a similar relationship. Inspection of Fig. 5 indicates that in the present stage of development of plastometric studies some convention must be adopted to select a single value of the static rigidity characterising a given soil. The most logical course would be to select the concentration corresponding to zero static rigidity, but the experimental error over this region becomes important, while extrapolation of the curve to the concentration axis is also subject to error. It appears better to adopt interpolation and to select that con-

centration at which any given material shows a static rigidity of a convenient constant value, *e.g.* one unit. This interpolation gives the moisture content at which the soil shows a definite degree of rigidity and is therefore analogous to the sticky point, differing only in the degree of rigidity chosen and in the method of measuring it. Among the small number of soils so far investigated, a close correlation exists between the two factors, except in the case of materials that are known to give anomalous behaviour in one or other of the measurements. Data for four normal soils are given in Fig. 6.

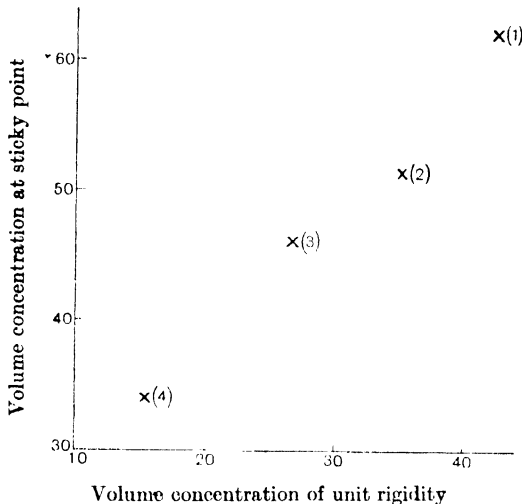


Fig. 6. (1) Punjab soil. (2) Beaumont alluvial subsoil. (3) Park grass, Rothamsted. (4) Fiji lateritic soil.

#### SUMMARY.

The laboratory study of the physical properties of soil and clays can conveniently be divided into three stages:

- (a) Moisture content comparable to that under field conditions.
- (b) Thick pastes.
- (c) Weak suspensions.

The use of the plastometer for experimental work on intermediate stage is described and recent developments of the theory of the flow for thick pastes under stress are outlined. It is shown that certain constants defining the material can be obtained from the experimental data. The two to which special attention is given are the pseudo-viscosity (a quantity analogous to the viscosity of true fluids) and the static

rigidity (which represents the energy required just to cause the paste to flow and a measure of the solid cohesive properties of the system). The latter quantity is related to other physical measurements made under very different experimental conditions, *e.g.* the resistance of the soil to the passage of cultivation implements; the effect of chalk, etc., on the soil resistance; the moisture content at which a well-kneaded mass of soil is about to become sticky.

## REFERENCES.

- (1) KEEN and HAINES. *J. Agric. Sci.* (1925), **15**, 375-86, 387-94, 395-406. (*Rothamsted Memoirs*, **12**.)
- (2) ——— *Proc. Inst. Int. Congr. Soc. Soil Sci.* (1927), **1**, 405-11.
- (3) HAINES. *J. Agric. Sci.* (1925), **15**, 178-200. (*Rothamsted Memoirs*, **12**.) (1927), **17**, 264-90). (*Rothamsted Memoirs*, **13**.)
- (4) SCOTT BLAIR and CROWTHER. *J. Physical Chem.* (1929), **33**, 321-30.
- (5) SCHOFIELD and SCOTT BLAIR. *J. Physical Chem.* (in the press).
- (6) HATSCHEK. *The Viscosity of Liquids*. G. Bell and Son (1928), 209.
- (7) BUCKINGHAM. Testing Materials. *Proc. Amer. Soc.* (1921), 1154.
- (8) REINER. *Kolloid* (1926), **39**, 80-87.
- (9) BINGHAM. *Fluidity and Plasticity*. McGraw-Hill Book Co. (1922).
- (10) KEEN and COUTTS. *J. Agric. Sci.* (1928), **18**, 740-65.
- (11) COUTTS. *J. Agric. Sci.* (1929), **19**, 325-41.

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# THE FLOW OF CLAY PASTES THROUGH NARROW TUBES

BY G. W. SCOTT BLAIR AND E. M. CROWTHER\*

After the classic equation of Poiseuille was put forward to account for the flow of fluids through narrow tubes, it was not long before various workers turned their attention to the modification which the equation would require in order that it might include within its scope those systems which are not true fluids, but have also some properties characteristic of solids. Trouton<sup>1</sup> for example, noted that the torsion-viscosity of certain "very viscous liquids" was not independent of the force of shear.

The first coherent attempt to produce a complete equation of flow for systems showing static rigidity was made by Bingham and Green.<sup>2</sup> These workers showed that a certain amount of pressure was required to overcome static rigidity, and that under suitable conditions, the Poiseuille equation could be applied, provided that the pressure term was taken as an excess over that amount required just to overcome the static rigidity. They pointed out, moreover, that flow curves plotted with the ordinates, "Amount of flow in unit time" and "Shearing stress applied" give for capillaries of different dimensions a family of straight lines, which, when extrapolated backwards over the region of low pressure (where the equation is not claimed to hold) all cut the "shear"-axis at a single point. This point gives a measure of the static rigidity of the system.

Bingham<sup>3</sup> points out that this linear relationship holds only at high rates of shear: "Fortunately, by using the higher rates of shear we can apparently always obtain the simple linear relationship. If later experiments prove that this is not the case, it will be time to use the more complex formulas." The constant corresponding to viscosity in the original Poiseuille equation, has been called by most workers by the unfortunate name of "Plasticity" and has the same dimensions as viscosity, although of course, it is not independent of pressure. Owing to the many different ways in which "Plasticity" has been used, we propose to term this constant "Pseudo viscosity." The reciprocal of this value (analogous to fluidity) is known as "Mobility."

Further, since no word has hitherto been adopted to describe the state of a substance exhibiting both static rigidity and fluid properties, we propose the term "pachoidal" with the corresponding noun "Pachoid" (Greek *παχὺς* thick). This would include substances showing the above pseudo-viscosity or Bingham's plasticity and also de Waele's plasticity, (see below) should this prove to be a different phenomenon.

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<sup>1</sup> Proc. Roy. Soc., 77A, 426 (1906).

<sup>2</sup> Bingham & Green: Proc. Am. Soc. Test. Mats., 1919, 641; Green: 1920, 450; Bingham: "Fluidity and Plasticity" (1922), and many papers.

<sup>3</sup> "Fluidity and Plasticity," 226 (1922).

Many American workers, especially ceramists, have made practical use of such high shear measurements, and have generally agreed with Bingham that conditions under low shears are too involved to justify serious investigations at present. (See especially Shearer.<sup>1</sup>)

Reiner<sup>2</sup> has treated the whole question from the theoretical point of view basing his work on the postulate of a critical shearing stress which must be exceeded before plastic flow can start at any point and taking into account the fact that this critical stress is not attained at different distances from the centre of the tube at the same pressure but is attained first at a point nearest the wall. He accounts for the curved portion in the lower regions of the flow curve by considering the solid geometry of the "Extrusion figures" when the critical shearing stress has been attained only in part of the cross-section of the tube, so that the material is flowing as a solid plug within an envelope which is being sheared.

Buckingham<sup>3</sup> had previously arrived at a similar equation, but had also taken account of the fact that at low pressures, before the critical shear has been reached, the system tends to flow through the tube as a plug, surrounded by a truly fluid envelope which is thin, and adheres to both the wall, and the plastic material. This treatment leads to a flow curve of three portions: (1) a straight line passing through the origin (plug flow); (2) a curved portion (combined plug and plastic flow) approaching asymptotically (3), a final straight line (plastic flow).

As a final equation of flow, he gives,

$$V = \frac{\pi R^4 \mu}{8L} \left( P - \frac{4}{3}p + \frac{p^4}{3P^3} \right) + \frac{\pi R^3 \epsilon \Phi P}{2L}$$

where:—

V is volume of flows in ccs. per second\*

R is radius of the capillary

$\mu$  is mobility

L is length of capillary

P is pressure applied

$\epsilon$  is thickness of liquid film through which plug flows

$\Phi$  is fluidity ( $1/\text{Viscosity}$ ) of this liquid film

p is pressure corresponding to critical shearing stress (f).

\* Buckingham actually prefers to write " $v/t$ " where  $v$  is the volume flowing in  $t$  secs.

The equation is qualified by the conditions that "the last term vanishes when there is no slip at the wall, and the first is to be omitted when  $P < (2f/R) = p$ ." Buckingham appears to have advanced no experimental evidence for this theory.

De Waele,<sup>4</sup> finding that experimental flow curves are parabolic rather than linear, has questioned the whole treatment, and has shown that his

<sup>1</sup> J. Am. Ceramic Soc., 11, 542 (1928).

<sup>2</sup> Kolloid-Z., 39, 80 (1926), and other papers.

<sup>3</sup> Proc. Am. Soc. Test. Mats., 1921, 1154.

<sup>4</sup> Oil and Colour J., 1923, 33; 1927, 232; J. Am. Chem. Soc., 48, 2760 (1926); Kolloid-Z., 36, 332 (1925).

equation, in which pressure is set as proportional (over a wide range) to a power of the flow, is valid at the very high pressures used by him. Many other workers (chiefly of the Ostwald school) claim that such an equation fits well the experimental facts at much lower rates of shear, and Green has pointed out that such a result would be expected on the Green-Buckingham theory if the experiments were carried out at insufficiently high rates of shear for the material investigated. It will be shown elsewhere that the results of the Ostwald school, when subjected to an alternative—and simpler—form of treatment, support the views to be given in the present paper.

In this paper the authors propose to give: (1) quantitative evidence to show that an equation only slightly modified from that of Buckingham holds under certain conditions of shear; and (2) qualitative evidence in favour of the Buckingham theory for those regions where quantitative measurements have not been practicable. This evidence has been obtained from experiments on the behaviour of certain clays (chiefly agricultural) in a modified Bingham plastometer.

### The Apparatus

The plastometer used in this work is essentially that used and described by Bingham and Green, the only modifications of importance being: (1) that there are two bulbs, the clay being sheared alternatively from one to the other; (2) that the level of clay in the two bulbs is kept the same so that no correction is required for hydrostatic head.

The air is forced by a foot pump into a reservoir, being released through a sensitive valve. When the clay paste is flowing under constant air pressure as measured on a mercury or water manometer, the air displaced by the clay is allowed to escape through one of a series of capillaries selected of a size convenient for the velocity to be measured. The extremely small pressure behind this capillary (proportional to the amount of air flowing through it) is read on a sensitive alcohol manometer inclined at an angle of about 1:10. To obtain the velocity of flow in  $\text{cm}^3/\text{secs}$ , the alcohol manometer readings need only be multiplied by a constant dependent on the dimensions of the air-capillary and determined by measuring the times required to shear a known volume of glycerine at constant rates. The pressures used varied from 1 mm. water to nearly 50 cm. Hg. and the rates of flow could be measured with accuracy from 0.0002 — 2.0  $\text{cm}^3/\text{secs}$ . The whole apparatus was enclosed in a thermostat at 25°C.

It will be seen from Fig. 1 that the apparatus is not geometrically regular. In order to be sure that the flow of a true fluid through the apparatus was closely in accordance with Poiseuille's law, experiments were done with glycerine, kerosene, and water, and the flow curves obtained were found to be good straight lines passing through the origin.

Although satisfactory results are obtained with natural clays and most soils, from which only the coarse sand has been removed, all the data given in this paper were obtained with separated clay fractions. Unless otherwise stated, the clay fractions were prepared from a heavy clay taken at a depth

of 3 feet below Broadbalk Field, Rothamsted. All the clay fractions were prepared by making a suspension in dilute sodium carbonate and decanting the top 8.5 cm. after sedimentation for 24 hours. The stable suspensions from a number of such repeated decantations were flocculated with dilute acetic acid, and filtered. The flocculated clay was washed with distilled water,

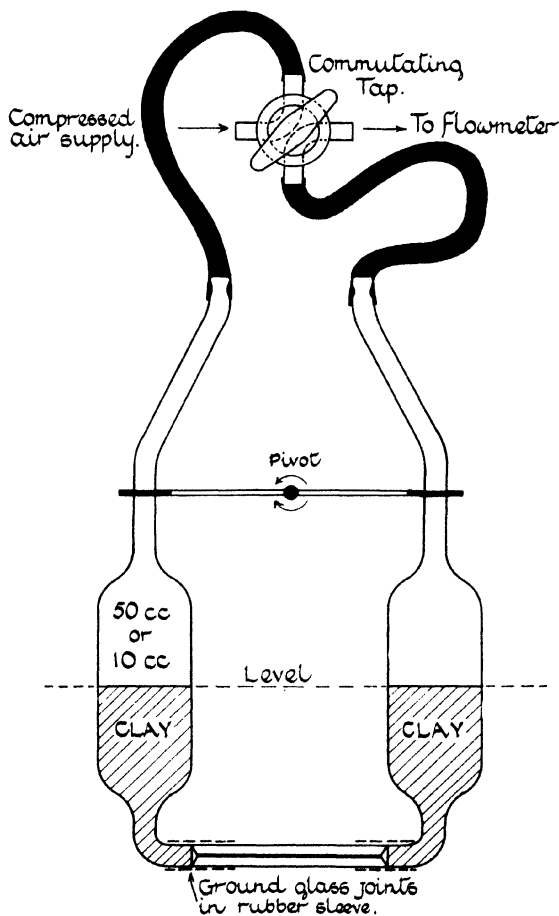


FIG. 1  
Plastometer Bulbs and Capillary

thoroughly mixed and bottled. For each series of experiments, a quantity of the paste was gently rubbed once or twice through a wire sieve with apertures of 0.2 mm. side. All comparisons are between samples taken from a single batch; separate batches were used for Tables I, II and III. The concentration figure given is the number of grams of dry matter in 100 grams wet clay after drying for one hour at 165°C.

The bentonite used for the dyed clay experiments was a commercial sample kindly given by Mr. A. de Waele. It was made up directly with dis-

tilled water without preliminary purification. It was then passed once more through the wire sieve. The dyed bentonite was washed until the wash-water was quite colourless.

For high shears, about 40 cc. of clay paste were sucked into the plastometer bulbs having a capacity of about 50 cc. each, and for low shears, about 8 cc of paste were used with bulbs of about 10 cc capacity. For high rates of flow a single reading, and for low rates, as many as six readings were obtained from each shearing from bulb to bulb. Under all the conditions considered in this paper, the number of shearings to which the clay had been subjected had no effect on the flow curve. Duplicate samples gave closely concordant results.

### The Experimental Flow Curves

A typical flow curve is given in Fig. 2. Owing to the wide range of rates of flow, the curve is divided into two portions with rates given in  $\text{mm}^3/\text{secs}$  and  $\text{cm}^3/\text{secs}$ . respectively. It was generally found convenient to use different capillaries for exploring these two portions of the curve, but, for simplification, in presentation, the data in Fig. 2 are taken from experiments on a single capillary. It was of course necessary to change the air-capillary of the flow meter in order to cover such a wide range. Even smoother curves than those in Fig. 1 were obtained when different capillaries were used for the two portions.

It is at once apparent from this figure that there are four separate stages in the behaviour of a clay paste subjected to increasing pressures. These are indicated in the diagram, and will be discussed separately.

Curves of the same type have been obtained for all the clays studied with the exception of kaolin, which gave serious irregularities due in part to seepage. In certain cases, as with clays of the bentonite type, stage II covered only a small range of pressures. After the removal of the coarse sand fraction, most soils gave curves similar to, and as regular as those of clays, but the concentration had to be much more carefully chosen.

On increasing the pressure gradually a number of different phases or regimes were obtained on the flow pressure diagrams.

*Stage I. There was no motion until a well-defined critical shearing stress was reached.*

In order to be certain that the initial point of flow was really sharply defined, a pressure of only a few per cent less than the observed critical pressure was applied, while the flow-meter air-capillary was completely blocked up. No rise at all was noted although the merest movement would have given a very large displacement in the flowmeter; the addition of an extra 1-2 mm. Hg pressure gave a definite reading with the unblocked air-capillary.

It will be seen from Table I that for flow in long narrow plastometer capillaries (with  $L/R$  greater than about 110) the values obtained by multiplying the pressure of initial flow (a) by the ratio  $R/L$  gave a constant (A) which was independent of the size of the capillary. For shorter and wider

tubes, this constancy did not hold. Further, for long and narrow capillaries the constant A was independent of the shape and size of the container bulbs, but with shorter and wider capillaries, as the ratio  $L/R$  was reduced below about 110, the critical pressures for initial flow began to show variations with changes in the form of the containers and approximate calculations indicated that the resistance of the bulbs themselves became significant.

If we follow Buckingham in assuming a plug flow at low shears through an envelope of constant thickness, the existence of a critical shearing stress for the start of the flow, can only be interpreted by assuming either (1) that the

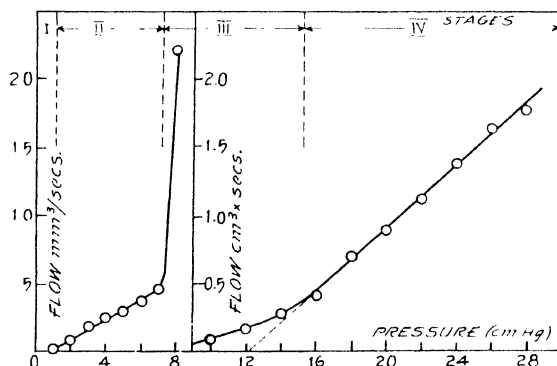


FIG. 2

A Typical Flow Curve

Clay paste 18.1%. Capillary No. 7.  $R = 0.060$  cm.  $L = 10.60$  cm.

fluid layer comes into being suddenly when a definite stress is reached, and attains at once its constant thickness or (2) that the layer is not truly fluid, but itself exhibits static rigidity or (3) that some bonds or forces exist across the fluid layer (already present) which are broken down on the attainment of a certain critical shearing stress.

TABLE I

Stage I. Constancy of critical shearing stress (A) for long narrow capillaries.

Capillaries for which  $L/R < 110$ , or  $R/L > 0.09$  are marked\*

Tube No.	Radius cm.	Length cm.	$10R/L$	Initial flow pressure cm. Hg "a"	Critical shearing stress. 10.(A)
2*	0.102	8.00	0.128	1.4	.18
8*	0.087	7.20	0.121	1.3	.16
3*	0.100	10.70	0.094	1.4	.13
6	0.088	10.65	0.083	1.5	.12
5	0.086	13.00	0.066	1.5	.10
13	0.049	8.00	0.061	1.6	.10
7	0.060	10.60	0.057	2.1	.12
11	0.049	10.00	0.049	1.9	.09
10	0.050	10.60	0.047	2.1	.10
12	0.049	12.90	0.038	2.7	.10

Alternative (3) seems the most probable, but the matter requires further investigation. The fact that readings can be taken repeatedly on both sides of the initial flow pressure indicates a reversibility which makes alternative (1) seem improbable.

*Stage II. In this stage the clay paste moves as a rigid plug through the tube and the flow curve is linear over a considerable range.*

Fig. 3 shows a photograph of a tube in which a paste of bentonite dyed fast with crystal violet was pressed through a capillary containing natural white bentonite of approximately the same moisture content. The sharp line of demarkation indicates a true "plug" flow.

Over this stage, the experimental points fall on a straight line within the limits of experimental error.

Table II shows as an empirical fact that the slope ( $dv/dP = x$ ) multiplied by  $L/R^3$  gives a constant ( $a$ ) which is independent of the dimensions of the capillary, provided that  $L/R$  exceeds about 50. The significance of this in its bearing on Buckingham's theory is dealt with below.

TABLE II  
Stage II. Constancy of  $X$  ( $= xL/R^3$  where  $x$  is slope) for different capillaries

Tube No.	Radius (cm.)	Length (cm.)	$L/10^4 R^3$	$x$	$10^{-4} X$
4	0.132	10.80	0.47	1.8	0.85
3	0.100	10.70	1.07	0.80	0.86
8	0.087	7.20	1.10	0.82	0.90
6	0.088	10.65	1.56	0.60	0.93
7	0.060	10.60	4.92	0.18	0.88
10	0.050	10.60	8.49	0.11	0.90



Fig. 3

*Stage III. The clay flows as a central plug within a stream-line shell of increasing thickness and the flow curve bends rapidly upwards.*

Qualitative evidence of the dual type of flow was obtained by a further experiment with dyed bentonite. Fig. 4 shows the cross-section of a capillary tube after the stress had reached the critical value, so that the dyed bentonite was now advancing as a central plug through a residual ring of undyed bentonite. That the dyed bentonite represents a plug rather than part of a stream-line system is indicated by the fact that the flow curve has a definite curvature.

*Stage IV. For the highest rates of shear studied the flow curves become linear and the flow is streamline throughout.*

It is found that the slope of the flow curve ( $y$ ) multiplied by  $L/R^4$  gives a constant ( $Y$ ) independent of the capillary.

When the flow curves are extrapolated to the pressure axis, the intercepts ( $c$ ) multiplied by  $R/L$  give a constant ( $C$ ) independent of capillary. (Table III)

TABLE III

Stage IV. Constancy of  $C$  ( $= cR/L$  where  $c$  is extrapolated intercept) and  $Y = (yL/R^4$  where  $y$  is slope) for different capillaries.

Tube No.	$10R/L$	$L/10^6R^4$	$C(\text{cm Hg})$	$C$	$y$	$10^{-6}Y$
3	0.094	0.107	7.5	.71	7.5	.80
6	0.083	0.178	8.5	.70	4.7	.84
7	0.057	0.820	11.7	.67	1.1	.90
10	0.047	1.70	14.2	.67	0.50	.85

(Slopes are expressed in the same arbitrary units throughout.)

### (C) The Linear Portions of the Flow Curves

Further evidence of the essential physical difference between the types of flow over the linear portions of the flow curves in Stages II and IV was obtained by experiments in tubes which had been roughened internally by means of a mixture of ammonium fluoride and hydrofluoric acid. The roughening of the capillary would be expected to interfere seriously with the thin envelope through which the plug flows in Stage II but to have little or no effect on the streamline flow of Stage IV.



FIG. 4

Fig. 5 gives the flow curves in both stages for a smooth and a roughened capillary of approximately the same diameters, together with curves calculated from the constants of the smooth tube for a tube of the same dimensions as the roughened one. In the streamline regime (Stage IV.) the experimental points for the roughened tube approximate to the calculated curve. But in Stage II there is a wide divergence and the rate of flow for a given pressure is greatly reduced. Further the flow curves were often more irregular than in the example given, indicating that the envelope was not only considerably reduced in thickness but was also less uniform.

The constants ( $X$  and  $Y$ ) derived from the slopes in Stages II and IV (Tables II and III) were found empirically to be connected, in that the ratio  $X/Y$  for a wide range of concentrations of the same clay was constant within the limits of experimental error. Clays of widely different geological types gave slight but significant differences in values of  $X/Y$ . (Table IV).

Such a relationship between  $X$  and  $Y$  was indicated in the work of St. John<sup>1</sup> who made comparisons with flour suspensions between a low-shear plastometer of his own design and the Bingham high-shear plastometer. Although St. John regarded both cases as instances of streamline flow, it seems probable that the low shear apparatus was giving a plug flow and that

<sup>1</sup> Ind. Eng. Chem., 19, 1348 (1927).

the correlations obtaining between the two sets of results for different flours depend on a somewhat similar relationship between  $X$  and  $Y$ .

A correlation of certain of the above plastometric constants for soil pastes with agricultural factors, such as the resistance of the soil to the plough, has been found and will be discussed elsewhere. It may be mentioned that some soils showed visible "seepage"\* and a consequent failure to give reproducible results on repeated shearing. Experiments are also in progress on the plastometric behaviour of clays and soils, with special reference to flocculation, exchangeable bases, and the effect of lime and fertilisers on soil tilth.

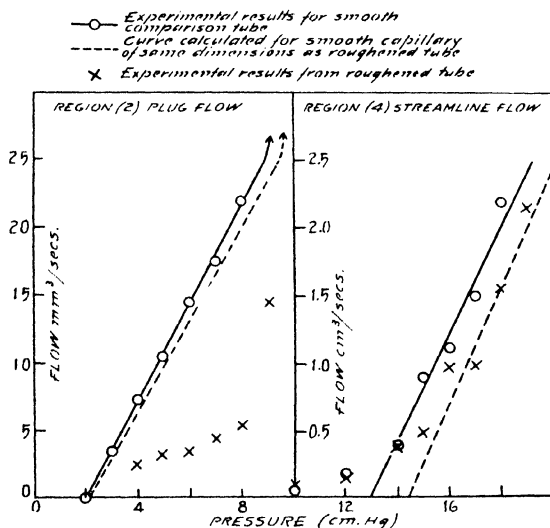


FIG. 5  
Effect of Roughened Capillary Surface on Flow Curves

TABLE IV

Values of Ratio  $X/Y$  for different concentrations of three clays

Clay A. Clay from Broadbalk Field, Rothamsted

Clay B. Oolitic Clay

Clay C. Liassic Clay.

Clay A		Clay B		Clay C	
Concentration	$X/Y$	Concentration	$X/Y$	Concentration	$X/Y$
21.9	2.2	24.8	1.5	24.5	1.7
19.3	1.8	23.8	1.5	20.8	1.7
18.4	2.0	19.5	1.5	18.3	1.4
18.2	2.2	18.2	1.1	16.4	1.6
15.5	2.3	17.2	1.6	12.6	2.0
14.3	2.0	14.6	1.0		
11.8	2.1	13.7	1.5		
10.7	2.3				
Mean	2.2		1.4		1.7

\* "Seepage" is defined as the condition in which the liquid suspension medium flows more rapidly than the bulk of the solid matter.

### Modification of Buckingham's Equation

Although the experimental data presented are not sufficient to test the whole range of Buckingham's equation, the constants derived from the two linear portions are in agreement with it, provided that it is modified to cover the initial region of no movement. This involves altering his assumption that the lubricating envelope acts as a true fluid for very low pressures. It is necessary to assume that a critical shearing stress must be obtained before any motion takes place and to deduct the equivalent pressure before applying the equation. This new shearing stress must not be confused with Buckingham's critical shearing stress ( $f$ ), which refers to the beginning of streamline flow in the paste itself (Stage III).

The above modification in the hypotheses in Buckingham's equation would lead theoretically to the expression

$$v = \frac{\pi R^4 \mu}{8L} \left( P - \frac{4}{3} p + \frac{p^4}{3P^3} \right) + \frac{\pi R^4 \epsilon \phi (P - a)}{2L}$$

The relationships between this expression and the constants derived from the above experimental data are as follows,

Stage I and II.  $a$  = the pressure equivalent to the critical shearing stress.

Stage III  $\epsilon \phi = x = (L/R^3)$ . slope.

Stage IV  $4/3P = c$  (= extrapolated intercept.)

and  $\mu = Y = L/R^4$ . slope.

$1/Y$  = pseudo-viscosity

Our thanks are due to Dr. B. A. Keen for his interest and criticisms throughout the progress of this work, and also to Mr. A. de Waele for advice in the earlier stages, especially in connection with the design of the plastometers.

### Summary

(1) A modified plastometer of the Bingham and Green type has been devised, suitable for experiments on small amounts of clay and soil pastes over a range of applied pressure of from 0.05 to 50 cm Hg, and rates of flow of from 0.0002 to 2.0 cm<sup>3</sup>/secs.

(2) The flow curves fall into four stages. In the first stage there is no flow; in the second the value of  $dV/dP$  is constant; in the third it increases rapidly and in the fourth it is again constant. The movements are successively—plug flow, plug flow with stream line flow in an outer sheath, and entirely streamline flow.

(3) This behaviour is shown qualitatively by experiments with dyed clays and for the most part quantitatively from the constancy of the plastometric functions to agree with an equation slightly modified from Buckingham's theoretical equation.

(4) It is found as an experimental fact that the ratio of the constants derived from the slopes of the plug and streamline stages respectively is constant over a wide range of concentration for a single clay. For clays of widely different geological origin variations in this ratio are small, but probably significant.





# THE INFLUENCE OF THE PROXIMITY OF A SOLID WALL ON THE CONSISTENCY OF VISCOUS AND PLASTIC MATERIALS\*

BY R. K. SCHOFIELD AND G. W. SCOTT BLAIR

## Introduction

In attempting to derive an expression for the rate of flow of a viscous or plastic material through a straight narrow tube of uniform cross-section under a pressure gradient, it is usually assumed:

- (1) that each particle of the material moves with constant velocity in a straight line parallel to the axis.
- (2) that there is no slip at the wall of the tube.
- (3) that the velocity gradient at any point depends only on the shearing stress at that point.

Using these assumptions, it is shown below that, no matter how complex the relationship between velocity gradient and shearing stress (so long as the former is fixed when the latter is fixed), the volume extruded in unit time will depend, for a given stress at the wall of the tube, upon the cube of the radius. While this is true for fluids, and is also true or nearly true for thick pastes of soil and other minerals, it is found not to be generally true of such pastes when examined over an extended range of concentration. Discrepancies would occur if condition (1) were invalidated owing to turbulence; but reasons are given for considering it unlikely that turbulence is responsible for the effect. It is found that the mean velocity, instead of being proportional to the radius, is divisible into two parts, one proportional to it and the other independent of it. The second term apparently represents a velocity imparted to the bulk of the material by an excessive velocity gradient near the wall of the tube, suggesting that the proximity of a solid wall influences the consistency of these materials and causes a breakdown of condition (3). On subtracting the second term, the contribution made to the mean velocity by the flowing of the bulk of the material is presumably left, from which consistency constants relating to the material in bulk can be obtained independent of the dimensions of the tube.

## *Theoretical.*

If condition (1) of the introduction be granted, so that the particles are not accelerated, the stress,  $W$ , at the wall of a tube of length  $L$  and radius  $R$  to which a pressure difference  $P$  is applied is  $PR/2L$ ; while the stress,  $S$ , at a point  $T$  within the tube and distant  $r$  from the axis is  $Pr/2L$ . Consequently  $r$  can be expressed in terms of  $S$  thus:

$$r = R/W.S. \quad (1)$$

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If  $v$  be the velocity at  $T$ , we may write in accordance with condition (3)

$$dv/dr = -f(S).$$

Substituting the value of  $r$  given by equation (i), and integrating

$$v = R/W \int_0^w f(S) dS, \quad (ii)$$

if, in accordance with (2)  $v = 0$  when  $S = W$ . The flow  $dV$ , between  $r$  and  $r + dr = 2\pi dr.v$ . Substituting for  $r$  and  $v$  from equations (i) and (ii) and integrating,

$$\frac{V}{\pi R^3} = \frac{2}{W^3} \int_0^w S \int_0^w f(S) dS.dS \quad (iii)$$

From this it is clear that, for any given material,  $V/\pi R^3$  should depend only on  $W$  if the three conditions are fulfilled.

By making specific assumptions about the form of  $f(S)$ ,  $V/\pi R^3$  can be evaluated. Thus using the Maxwell assumption that

$$f(S) = \mu S$$

where  $\mu$  (the fluidity) is the reciprocal of the viscosity, equation (iii) reduces to Poiseuille's equation in the form

$$\frac{V}{\pi R^3} = \frac{1}{4} \mu W.$$

In the same way the expression based on the Bingham<sup>1</sup> assumption can be deduced. Here it is supposed that the material does not flow unless a stress exceeding a critical value,  $S_0$ , be applied to it and that, at stresses higher than  $S_0$ , the velocity gradient equals  $\mu(S - S_0)$ . Again  $\mu$  is a constant having the dimensions of a reciprocal viscosity, and is usually called the mobility. When such a material is forced through a tube, a central cylinder of radius  $RS_0/W$ , within which the stress is less than  $S_0$ , moves as a solid plug, and only the material outside this cylinder flows. When  $W$  is less than  $S_0$ , no flow occurs, and  $V = 0$ . In substituting in equation (iii) to obtain the value of  $V$  when  $W$  exceeds  $S_0$ , it must be remembered that, since  $f(S)$  is discontinuous, being zero from 0 to  $S_0$  and  $\mu(S - S_0)$  from  $S_0$  to  $W$ , the integrations must be carried out in two stages. This has the effect of splitting  $V$  into two terms, thus

$$\frac{V}{\pi R^3} = \frac{2}{W^3} \int_0^{S_0} S \int_{S_0}^w \mu(S - S_0) dS.dS + \frac{2}{W^3} \int_{S_0}^w S \int_{S_0}^w \mu(S - S_0) dS.dS.$$

The first is the contribution of the plug, the second is that of the flowing material between it and the wall. This reduces to

$$\frac{V}{\pi R^3} = \frac{1}{4} \mu \left[ W - \frac{4}{3} S_0 \left\{ 1 - \frac{1}{4} \left( \frac{S_0}{W} \right)^3 \right\} \right]$$

which is subject to the condition that  $W > S_0$ . This is equivalent to the

<sup>1</sup> E. C. Bingham: "Fluidity and Plasticity," (1922).

equation<sup>1</sup> deduced by Buckingham,<sup>2</sup> and independently by Reiner.<sup>3</sup> The graph connecting  $V/\pi R^3$  and  $W$  corresponding to this equation is tangential to the  $W$  axis at  $W = S_0$ . It is strongly curved for values of  $W$  only slightly exceeding  $S_0$ , but at higher values approximates to a straight line of equation

$$\frac{V}{\pi R^3} = \frac{1}{4} \mu (W - \frac{4}{3} S_0).$$

This line makes an intercept on the  $W$  axis equal to  $4/3 \cdot S_0$ , and, like the corresponding graph of the Poiseuille equation, has a slope of  $1/4 \cdot \mu$ . The true curve is steeply asymptotic to the limiting straight line, the discrepancy in  $V$  being less than 1% when  $W$  exceeds 2.2 times the intercept of the limiting straight line.

If the parabolic relation

$$f(S) = \bar{\mu} S^n$$

of the Ostwald type<sup>4</sup> be assumed, equation (iii) reduces to

$$\frac{V}{\pi R^3} = \frac{1}{n+3} \cdot \bar{\mu} \cdot W^n$$

which is equivalent to the equations given by Farrow, Lowe and Neale,<sup>5</sup> and Porter and Rao.<sup>6</sup> The constant  $\bar{\mu}$  has dimensions which depend on the exponent  $n$ . The graph in this case starts from the origin, and is curved throughout its length. The curvature is never very strong, but it decreases only slowly with increasing  $W$ .

### Experiments.

The modified Bingham plastometer used in this work has already been described.<sup>7</sup> The paste to be investigated is made by mixing the soil, clay or other mineral with water into a smooth paste, which is then forced through a one hundred mesh-per-inch sieve to remove any coarse particles. The paste is then diluted to the required concentration, and sucked into the plastometer bulbs which, for the high-stress work here described, have a capacity of 100 c.c. each. The material is forced alternately from one bulb into the other through one of a series of standardised tubes, the level in the bulbs being kept approximately the same by tilting the whole system about a pivot. For this more accurate work with larger bulbs it becomes necessary to correct the pressures for the resistance offered by the bulbs themselves. For this purpose the bulbs are connected directly with one another, and the pressures corresponding to a series of volume-flows are measured. By graphi-

<sup>1</sup> Buckingham's equation contains an additional term which is negligibly small when  $W$  exceeds  $S_0$ , and which is referred to below.

<sup>2</sup> E. Buckingham: *J. Am. Chem. Soc., Test. Mat.*, **1921**, 1154.

<sup>3</sup> Reiner: *Kolloid-Z.*, **39**, 80 (1926), etc.

<sup>4</sup> Wo. Ostwald (and others): *Kolloid-Z.*, **36**, 99, 157, 248 (*Zsigmondy Festschrift*) 252 (1928); **38**, 261 (1926); **41**, 56, 112 (1927). (This type of equation was, of course, not originated by Ostwald, but he has made much use of it.)

<sup>5</sup> Farrow, Lowe and Neale: *J. Textile Inst.*, **19**, T 18 (1926).

<sup>6</sup> Porter and Rao: *Trans. Faraday Soc.*, **23**, 311 (1927).

<sup>7</sup> G. W. Scott Blair and E. M. Crowther: *J. Phys. Chem.*, **33**, 321 (1929).

cal intrapolation the correction,  $P_b$ , corresponding to each volume-flow can be estimated. As no appreciable increase in resistance is caused by introducing a few millimeters of narrow tubing between the bulbs, it may safely be concluded that no kinetic energy correction is necessary with these measurements. The tubes had been carefully selected with a view to uniformity of bore and were standardised by weighing the quantity of mercury required to fill them. A series of constant pressures are applied by means of compressed air, the pressure being measured on a water or mercury manometer according to its magnitude. The air displaced by the clay is allowed to escape through an air-capillary of suitable dimensions. The pressure difference (negligible in comparison with the applied pressure) is measured on an alcohol manometer at an angle of one in ten (the flow-meter), and is directly proportional to the volume of flow of paste per second. The moisture content of the paste is determined by heating a sample for one hour in an oven at a temperature of  $160^{\circ}\text{C}$ . The concentration,  $K$ , is expressed as the number of grams of dry matter per 100g paste. Volume concentrations are calculated on the basis of a constant specific gravity for the dry material of 2.7.

In carrying out this work efforts have been made to use as wide a range of radii as possible. Although it is hoped in the future to increase this still further, difficulties will first have to be overcome. Thus the use of very wide capillaries involves large volumes of material and consequently big bulb correction (always difficult to determine accurately). Moreover the increased length that must be given to the tube necessarily entails a sacrifice of uniformity in the bore. Thus beyond certain limits, the loss in accuracy renders further increase in radius of no advantage. With very narrow tubes so coarse a system as a soil-paste behaves erratically.

In Table I are given, as an example, the complete data for a sample of Broadbalk Field subsoil similar to that of which the clay-fraction has been used in the previous work.

TABLE I

Plastometric data for Broadbalk subsoil paste (33.5 g dry soil per 100 g paste).

Cap. II.  $\dot{R} = 0.093 \text{ cm.}$   $L = 12.20 \text{ cm.}$

P	a	V	$P_b$	S	v.
8.0	3.0	2.0	1.1	3.5	.75
9.0	4.2	2.7	1.3	3.9	1.05
10.0	4.4	3.0	1.4	4.4	1.10
11.0	5.2	3.5	1.5	4.9	1.30
12.0	5.7	3.7	1.5	5.4	1.42
13.0	6.7	4.6	1.8	5.7	1.68
14.0	7.0	4.8	1.8	6.2	1.75
15.0	8.2	5.4	1.9	6.7	2.05

Cap. III.  $R = 0.073$  cm.  $L = 12.10$  cm.

P	a	V	Pb	S	v.
14.0	2.8	1.9	1.0	5.2	1.13
16.0	3.7	2.5	1.3	5.9	1.49
20.0	4.2	2.9	1.4	7.4	1.73
22.0	5.1	3.5	1.4	8.2	2.08
24.0	5.7	3.9	1.5	9.0	2.32
26.0	6.1	4.2	1.6	9.7	2.50
28.0	6.8	4.6	1.8	10.4	2.74
30.0	7.3	5.0	1.8	11.2	2.97

Cap. 7  $R = 0.059$  cm  $L = 10.60$  cm

P	a	V	Pb	S	v.
15.0	1.5	1.0	0.7	5.3	0.93
20.0	2.4	1.6	1.0	7.1	1.48
25.0	3.2	2.0	1.1	8.9	1.98
30.0	4.0	2.6	1.3	11.1	2.50
35.0	4.8	3.1	1.4	12.5	3.00

Cap. IV.  $R = 0.048$  cm.  $L = 12.25$  cm.

P	a	V	Pb	S	v.
16.0	0.5	.34	0.6	3.9	.47
24.0	1.05	.72	0.7	6.0	.99
28.0	1.3	.88	0.7	7.0	1.22
32.0	1.6	1.09	0.8	8.0	1.50
38.0	1.95	1.33	0.8	9.5	1.85
40.0	2.0	1.36	0.8	10.0	1.88
42.0	2.1	1.43	0.8	10.5	1.98
44.0	2.25	1.53	1.0	11.0	2.12

Cap. V.  $R = 0.040$   $L = 12.30$  cm.

P	a*	V	Pb	S	v.
20.0	0.3	.06	0.6	4.2	.40
25.0	0.5	.10	0.6	5.3	.66
30.0	0.7	.13	0.6	6.4	.93
35.0	0.85	.16	0.6	7.4	1.13
40.0	1.05	.20	0.6	8.5	1.35
45.0	1.2	.23	0.6	9.6	1.60

*Bulbs alone without capillary.*

(A water manometer was used, but P is given converted into cm. Mercury).

P 0.5 1.0 1.5 2.0

a \*0.2 0 2.0 6.0 8.0

V 0.4 0 1.4 4.1 5.4

\* ( $V/a = .19$ ).

P is pressure in cm. mercury.

a is flowmeter reading ( $V/a = 0.68$ ).V is volume flow in  $\text{cm}^3/\text{secs}$ .

Pb is pressure due to bulbs in cm. mercury.

S is stress in dynes/ $\text{mm}^2$  (calculated from  $P - \text{Pb}$ ).

v is mean velocity in metre/secs.

It is well known that the data obtained with such an apparatus when plotted on a  $V/\pi R^3 - W$  basis fall into two groups. In one, which may conveniently be called type A the points for a single tube lie, within the limits of experimental error, on a straight line passing through the origin. In the other

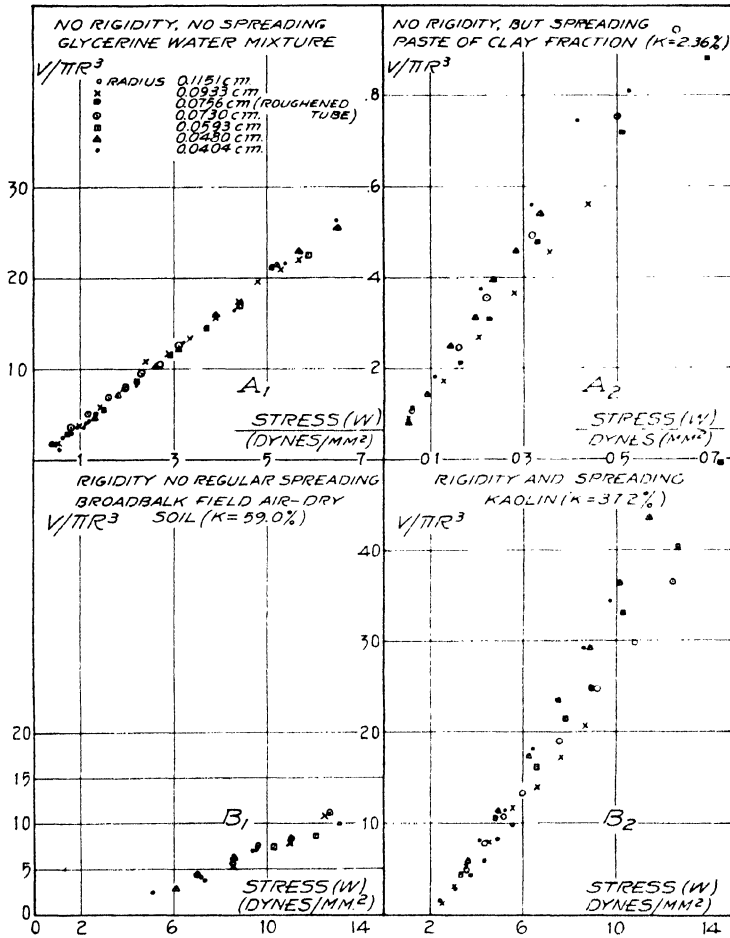


FIG. 1

Legends incorporated in four corners of each quarter of drawing.

(type B) this is not the case. Our own measurements not only confirm this fact but show that each group must be further subdivided according as the curve obtained is or is not independent of  $R$ . There are thus four possibilities, an instance of each which is given in Fig. 1.

With the water-glycerine mixture (type A<sub>1</sub>) a single straight line through the origin gives an adequate representation throughout the range of stress used. This is not true of the dilute suspension of very fine soil particles.

(type A<sub>2</sub>) Here the best line though passing through the origin has a larger slope the smaller the radius. Thus the disconcerting fact is here revealed that a straight line through the origin obtained with a single tube is not by itself a proof that Poiseuille's law is being obeyed.

The thick paste of Broadbalk Field surface soil gives points which, though more erratic than those for water-glycerine, show no regular trend with change of radius. Nevertheless they cannot be represented as falling on a straight line through the origin (type B<sub>1</sub>). The behaviour of *thick* soil pastes has already been described in detail in the earlier paper,<sup>1</sup> where it is shown that the curves can be interpreted in the light of the Bingham postulate. New and more accurate measurements over a wider concentration range has shown that a slight spreading noticeable in some of the earlier data and attributed to experimental error cannot be so explained. In thinner pastes of soils, clays and simple minerals such as barytes and gypsum the spreading is very marked. A kaolin paste of moderate consistency is given as an example (type B<sub>2</sub>). Here as with type A<sub>2</sub>  $V/\pi R^3$  for a given value of  $W$  increases as  $R$  decreases.

An alternative way is to regard type B<sub>2</sub> as the general case: the other three being special and simpler cases. Such a view raises the question as to whether all these systems are susceptible to the same treatment, and can therefore be represented by a single though complex, equation. Already in the interpretation of curves of the B<sub>1</sub> type, two distinct schools of thought have developed, one of which bases its treatment on Bingham's postulate and the other on the Ostwald postulate. This is not the place to enter into a general discussion of the relative advantages of the two methods, suffice it to say that all the soil and mineral pastes investigated in this laboratory are more amenable to the first method; and that although there undoubtedly are systems such as benzene-rubber and pastes of at least some starches that give curves of a shape not accounted for by the simple Bingham postulate, it is nevertheless true that much of the data which is represented as conforming to a relationship of the Ostwald type can as well be cited in support of the simple linear relationship. (Vide Herschel and Bulkley,<sup>2</sup> Porst and Moskowitz,<sup>3</sup> Scott Blair,<sup>4</sup> Ostwald<sup>5</sup>).

Hatschek<sup>6</sup> has criticised the practice of extrapolating flow-curves by means of straight lines, and considers that, failing a discontinuous change from a curved to a straight portion, the choice of the portion to be regarded as straight is arbitrary and a matter of scale. According to the Bingham treatment these straight lines are asymptotes to which the true flow-curve approximates more and more closely as the stress increases. It is clear in general that the error involved in drawing an asymptote to an experimental curve depends on the

<sup>1</sup> G. W. Scott Blair and E. M. Crowther: *J. Phys. Chem.*, **33**, 321 (1929).

<sup>2</sup> Herschel and Bulkley: *Ind. Eng. Chem.*, **16**, 927 (1924) etc.; *Proc. Am. Soc. Test. Mat.*, **26**, 621 (1926).

<sup>3</sup> Porst and Moskowitz: *J. Ind. Eng. Chem.*, **14**, 49 (1922).

<sup>4</sup> Scott Blair: *Kolloid-Z.*, **47**, 76 (1929).

<sup>5</sup> Ostwald: *Kolloid-Z.*, **47**, 176 (1929).

<sup>6</sup> Hatschek: "The Viscosity of Liquids," 209 (1928).

steepness of approach. It would certainly be difficult to draw the asymptote to a rectangular hyperbola given only a portion of the curve; but the criticism loses its force where the curve is of the Buckingham-Reiner type. In this case, already noted, the discrepancy in  $V$  is less than 1% for values of  $W$  greater than 2.2 times the intercept. Above this limit the difference between the true curve and the limiting straight line should be outside the limits of experimental error. This is borne out in practice with soil and clay pastes, so that linear extrapolation appears justified with these materials.

### *Discussion.*

These experiments yield the result that for many viscous suspensions (type A) as well as plastic pastes (type B),  $V/\pi R^3$  does not depend only on  $W$ . As the variation of the former quantity sometimes approaches twofold for a twofold variation of radius, the effect is evidently quite outside the limits of ordinary experimental error. Moreover the fact that the apparatus gives a very satisfactory verification of Poiseuille's law for true fluids indicates that it works satisfactorily. The effect has every appearance of being genuine. The next step therefore is to seek its cause. This must lie in a breakdown of one or more of the three conditions set out in the introduction. These will be considered in turn.

A breakdown of the condition that the particles move with a constant velocity parallel to the axis would occur if the flow were turbulent. Although no complete theory of turbulent flow has yet been advanced, it is generally considered that its presence is marked by a falling off in the slope of the flow-curve as the stress is increased. Such a falling off does occur at very high stresses, particularly with the more dilute suspensions; but the curves obtained with a view to elucidating the effect under discussion were not followed far enough for this to happen, and as can be seen from the examples given, show no signs of curvature over the range examined. It might be urged that the close approximation to linearity arises from a chance cancellation of opposite curvations, in a manner similar to that suggested to explain the "Laminarast" or linear portion of the flow-curves obtained by Ostwald and Auerbach.<sup>1</sup> As against this, it should be pointed out that many hundreds of flow-curves for clay and soil pastes have now been accumulated in this laboratory, and not one reliable curve has been obtained which cannot be fairly represented by a straight line at sufficiently high stresses. It seems inconceivable that an exact cancellation of two unconnected tendencies should occur in all these cases. It is more reasonable to interpret the straightness as an indication that these materials are obeying both condition (1) and the Bingham postulate and to endeavour to deduce an equation of the Buckingham-Reiner type based on a modification of conditions (2) and (3).

Until the results to be expected when flow is turbulent have been more fully worked out, it is impossible to exclude it altogether from the possible causes contributing to the effect. All that can be said at present is that there

<sup>1</sup>Ostwald and Auerbach: *Kolloid-Z.*, **38**, 261 (1926); **41**, 56 (1927).

is no positive evidence that turbulence is present in these experiments. This statement applies with equal force to the 'structure' type of turbulence postulated by Ostwald as to the more general type.

On plotting the mean velocity  $V/\pi R^2$  rather than  $V/\pi R^3$  against  $W$ , a regularity becomes apparent. A typical set of curves plotted in this way

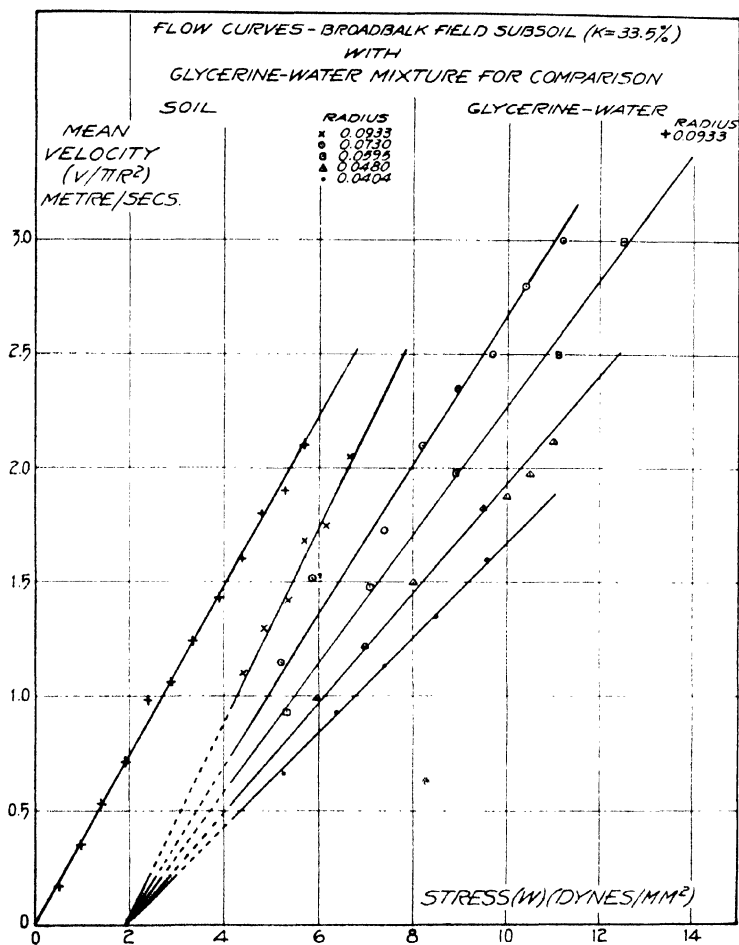


FIG. 2

from the data in the table is shown in Fig. 2. Since according to the Buckingham-Reiner equation (see above) the points should lie above the limiting straight line by more than 1% at values of  $W$  less than 2.2 times the intercept these have been omitted, and only those for stresses above 4.4 dynes/mm<sup>2</sup> have been used for locating the limiting straight lines. As these have a common intercept their slopes would be proportional to  $R$  were  $V/\pi R^3$  dependent only on  $W$ . Actually when the slopes  $\sigma$  are plotted against  $R$  a

straight line can in all cases be drawn through the points, but for types A<sub>2</sub> and B<sub>2</sub> this does not, when extrapolated, pass through the origin, but gives a positive intercept on the slope axis. Curves connecting  $\sigma$  and  $R$  which may conveniently be spoken of as derived curves are given in Fig. 3 for the four sets of curves of Fig. 1. The derived curve obtained from Fig. 2 together with ones for pastes of barytes and gypsum are given in Fig. 4. If turbulence

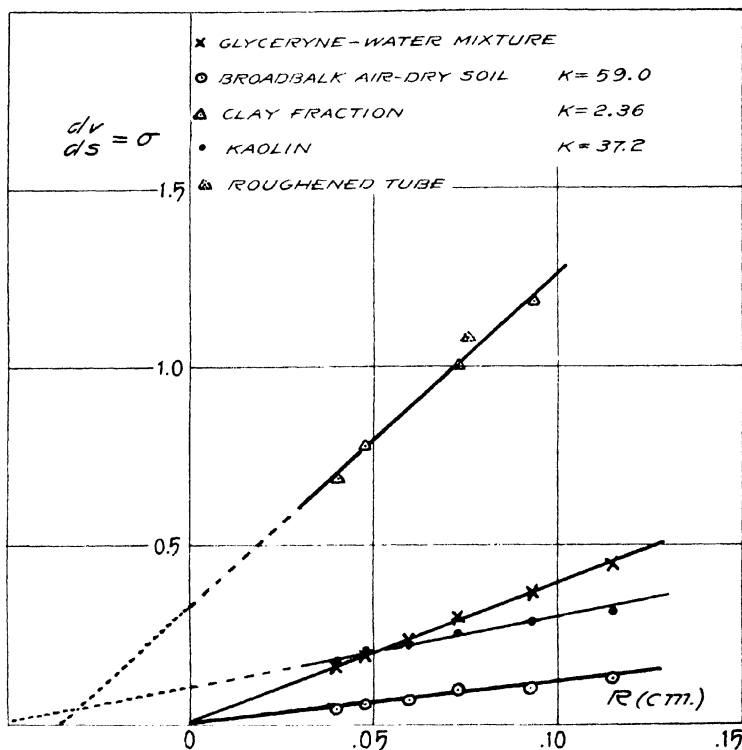


Fig. 3  
Derived curves

were the sole cause of the effect, the disturbance would presumably be most marked in the widest tube. In these conditions the mean velocity for a given stress on the wall should be more nearly proportional to the radius with the smaller tubes than with the wider ones. In other words the derived curve should approximate more closely to a straight line through the origin the smaller the radius. It will be seen that the curves in Figs. 3 and 4 taken as a whole do not support this idea.

If, on the other hand, the derived curves are in reality straight lines (as shown), the fact that some give an intercept might be interpreted as indicating that conditions (1) and (3) are fulfilled, but that in such cases there is a slip at the wall. The slope  $\sigma$ , and hence the mean velocity at a given value of  $W$  can in these cases be separated into two components, one proportional to

the radius and one independent of it. It seems more probable however that the second component instead of representing an actual slip at the wall should be regarded as a velocity imparted to the bulk of the material by excessive flowing of the material in the immediate vicinity of the wall. Provided that the thickness of the region in which this excessive flow takes place is both independent of the radius and also small in comparison with it, the effect on

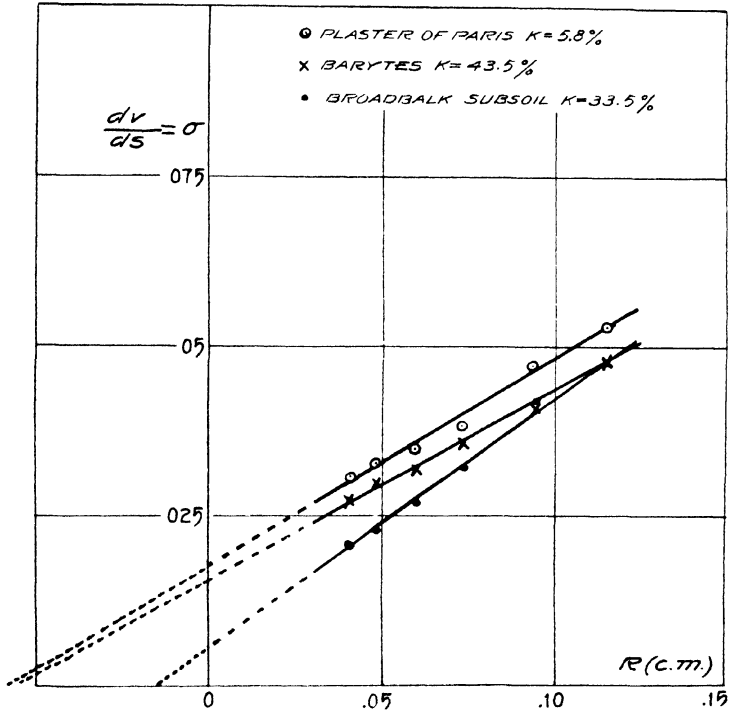


Fig. 4  
Derived Curves

the mean velocity would be the same as that of a slip at the wall itself. There would however be a difference in tubes so narrow that the radius is of the same order of magnitude as the thickness of the region of excessive flow, as in this case the derived curve would bend round towards the origin.

The first component of the mean velocity, since it is proportional to  $R$  is presumably due to the flow of the material in bulk, and therefore equal to  $V/\pi R^2$  calculated from equation (iii) using the appropriate value for  $f(S)$ . In cases where the Bingham postulate is applicable to the material in bulk the first component will be that given by the Buckingham-Reiner equation and the slope of the derived curve will equal  $\frac{1}{4}\mu$ . The second component of mean velocity is equal, according to the constructions in Figs. 2 and 3, to  $\sigma_0 (W-C)$  when  $\sigma_0$  is the intercept of the derived curve on the  $\sigma$  axis and  $C$

the common intercept of the flow curves (Fig. 2) on the  $W$  axis (which according to the Buckingham-Reiner equation should equal  $4/3 S_0$ )

The existence of a very thin layer of fluid of the consistency of water, separating the paste from the wall has been assumed by Buckingham to account for the small movement which occurs at stresses so low that the bulk of the material does not flow. This view, slightly modified, was adopted in the earlier paper, where it was shown experimentally that this flow is related to the stress thus:

$$V/\pi R^2 = v = \epsilon \phi (W - A) \quad (\text{vii})$$

$A$  being a constant stress below which no movement occurs,  $\epsilon$  the thickness of the layer and  $\phi$  its mobility. It might at first appear that the second component of the velocity is the same as the above. This, however, can hardly be the case since  $\sigma_0$  is found to have a magnitude some 100 times that of  $\epsilon \phi$ . For this reason the second component, unlike the Buckingham term, cannot be neglected at high stresses when the material is flowing. Assuming a value of  $\phi$  equal to the fluidity of water in bulk, the thickness  $\epsilon$  is of the order  $10^{-5}$  cm. The corresponding thickness calculated from  $\sigma_0$  is of the order  $10^{-3}$  cm. As the mobility of the modified layer cannot be greater than that of water in bulk and is probably less, this latter is a minimum estimate.

Experiments with tubes, the walls of which had been etched with fluoride also reveal an essential difference between the two phenomena. It was found (*loc. cit.*) that etching greatly interferes with the motion at very low stresses and renders equation (vii) inapplicable. No corresponding influence on  $\sigma_0$  is observed. Thus it will be seen from Figs. 1 and 3, that the points obtained with the etched tube fall into line with those for the smooth tubes. A further distinction is apparent in the behaviour of pastes made from soils that have previously been air-dried. Movement at low stress is inhibited whereas no similar interference is found at high stresses. It would appear therefore that, where the derived curve does not pass through the origin, there exists in the immediate neighbourhood of the wall of the tube a region in which the viscous or plastic properties of a material flowing through it are modified. When a laminated material such as a clay paste flows through a tube particles near the wall will tend to align themselves in the direction of flow, and be unable to rotate under the influence of the viscous couple acting on them. In the bulk of the material the particles will rotate sufficiently to prevent any alignment. If indeed there be any such an orientation near the wall it might give rise to an increase in mobility as the wall is approached and thus to a deviation from equation (iii) such as is actually observed. On the other hand attempts to eliminate the  $\sigma_0$  term by the use of materials which are believed to be devoid of laminar structure has so far proved unsuccessful. Only true fluids show both no  $\sigma_0$  term and no rigidity. An alternative explanation would involve an increased concentration of the suspended material towards the centre of the tube relative to the region near the wall, the relatively more dilute material having a greater mobility. In order to test this idea a sample of clay suspension giving a high value for  $\sigma_0$  was forced through a metal tube through

the side of which a hole had been drilled. This hole was very small so that the exuding of material through it would scarcely interfere with the flow in the tube. Although a large variation in concentration would have to be assumed to account for the value of  $\sigma_0$  observed, no appreciable difference in concentration was found between the exuded material and the rest.

We are not, therefore, in a position to offer a detailed physical explanation of the effect observed. Yet the view that the properties of the material are modified in the neighbourhood of the wall is supported by another fact which has been repeatedly observed, but which has hitherto received no explanation. It will be seen that in Fig. 2 of the previous paper (p. 326) the ratio of the extrapolated intercept on the pressure axis in the V-P curve, to the pressure at which flow at the wall just starts, is somewhat greater than the  $4/3$  necessitated by the Buckingham-Reiner equation. This discrepancy may well be due to a decreased value of  $S_0$  in the neighbourhood of the wall.

#### *The Determination of Consistency Constants.*

Methods for determining absolute consistency constants have hitherto been based on the supposition that, provided the motion is not turbulent,  $V/\pi R^3$  depends only on  $W$  and the nature and concentration of the material. Where this is not true (Types A<sub>2</sub> and B<sub>2</sub>) the methods require modification whether the constants are defined with reference to the curves obtained by plotting  $V/\pi R^3$  against  $W$  (Buckingham-Reiner equation) or by plotting the logarithms of these quantities (Farrow, Lowe and Neale equation). In such cases, it is impossible to define a viscous constant for the material from measurements made with a single capillary, since there would be a progressive change in its value with radius were the usual method adopted. An instance is given in Fig. 5 where the viscosity as given (1) by the Poiseuille-Bingham method for wide and narrow capillaries respectively and (2) from the slope of the derived curve is plotted against the concentration. The latter construction has the advantage of giving a single constant independent of radius, which, if the considerations advanced above are correct is a measure of the viscous properties of the bulk of the material.

Moreover, the shape of the viscosity-concentration curve as given by the second construction is such as would be expected if the deviation from linearity were due simply to a decrease in the extent of hydration of the particles. This shape is not reproduced in the curves derived from the single capillaries. The curve from the smallest capillary has an upward curvature of the type frequently obtained for concentration curves for hydrophylic materials with a simple Ostwald viscometer.

It is at least apparent that measurement of the viscosity of suspensions cannot be considered to be reliable unless made with at least two tubes of reasonably differing radii.

The method at present used in this laboratory for determining the consistency constants for soil and clay pastes is as follows:

The data for a series of four or five different capillaries having a total range of radius of at least two-fold are plotted out on a  $V/\pi R^2 \cdot PR/2L$  basis,

the values of  $P$  having previously been corrected for the resistance of the bulbs. The best straight lines are then drawn through the points in such a way that they converge on the stress axis. The point at which these extrapolations converge is taken as the rigidity,  $C$ .<sup>1</sup> The slope ( $\sigma$ ) of each curve is then measured (i.e. the rise in  $V/\pi R^2$  per unit increase in stress) and  $\sigma$

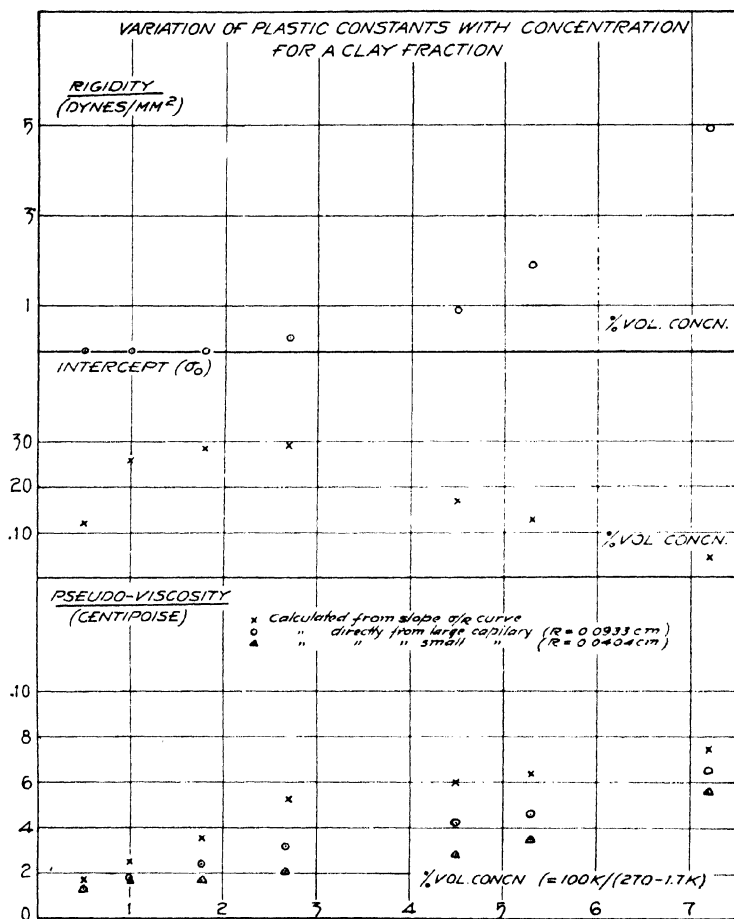


FIG. 5

is plotted separately against  $R$ . The slope of the derived curve ( $dR/d\sigma$ ) divided by 4 is taken as the viscous constant  $\eta'$  (pseudo-viscosity =  $1/\mu$ ) and the intercept of the extrapolated curve on the  $\sigma$  axis as  $\sigma_0$ , a measure of the wall effect.

Fig. 5 shows an interesting relationship for a clay fraction between  $\sigma_0$  and concentration, the value of  $\sigma_0$  passing through a maximum at a low

<sup>1</sup> This is, of course, the "limit of rigidity" i.e. Bingham's yield value, not the rigidity modulus.

concentration and disappearing for pure water and also at high concentrations where the high rigidity limits a further extension of the concentration range. Accurate measurements of  $\sigma_0$  at low concentrations are not easy, and the values are liable to a fairly large error, but there can be no doubt as to the general shape of the curve. It is of interest that the maximum of the curve occurs at about the same concentration as that at which rigidity first makes its appearance.

Since the dimensions of  $\sigma_0$  are somewhat inconvenient, an alternative method is to extrapolate the derived curves still further onto the negative radius axis. In this way a hyperthetical length ( $R_0$ ) is obtained which must be added to each radius before the equation stating the proportionality of the slope of the  $V/\pi R^2: PR/2L$  curves to (viscosity  $\times$  radius) can be applied. In other words  $R^3(R + R_0)$  takes the place of the  $R^4$  in the equations of Poiseuille or Buckingham-Reiner when written so as to give  $V$  directly in terms of  $P$ .

Our thanks are due to Dr. B. A. Keen for his interest and criticisms throughout the progress of this work.

### Summary

If, in considering the flow of a plastic material through a narrow tube, it be assumed that the velocity gradient at any point depends only on the stress at that point, it necessarily follows that the mean velocity for a given stress at the wall of the tube should be directly proportional to the radius of the tube. Although thick soil pastes conform closely to this requirement, thinner pastes whether they show rigidity or not give marked discrepancies. These discrepancies can be accounted for by assuming that in the immediate proximity of the wall a modification of the plastic properties occurs, which imparts an additional velocity to the bulk of the material. By first subtracting this velocity a viscosity constant is obtained independent of the dimensions of the tube.



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# FURTHER NOTE ON THE CAPILLARY FORCES IN AN IDEAL SOIL.

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IN a previous paper<sup>(2)</sup> the author has treated of the statical forces due to capillarity in an ideal soil. That paper was written in order to amend certain erroneous formulae for the static stress in such a soil, previously put forward by W. B. Haines<sup>(1)</sup>. These formulae covered only that range of water content in which the water exists in isolated rings surrounding the points of contact of adjacent soil particles, and after correction, both for the statical errors of the treatment, and for the geometrical approximation employed, it appeared that the cohesive stress ascribable to capillary forces falls off from its limiting value for dry soil at a rate of only a quarter of the rate found by Haines.

Haines had experimented with certain soil-like materials with the intention of measuring the static stress. His method was to cause a rupture in the soil aggregate by the intrusion of a steel wedge, and to ascribe the maximum resistance encountered to the static stress due to capillary forces. The values obtained varied from zero for dry ignited soil to a maximum near to saturation, at every stage increasing with increasing water content. These experimental values are shown on a diagram (1, Fig. 2); on the same diagram is shown a theoretical curve consisting of (1) a rapidly falling portion based upon the calculations referred to above; and (2) a rising portion curved in harmony with the experimental values and terminated by an abrupt fall at saturation. This latter portion of the theoretical curve seems to be wholly conjectural; an attempt is made in the text to justify its final value by two assumptions (1) that the static stress at saturation may be equated to the pressure deficiency in a liquid filling the soil pores, and (2) that this pressure deficiency will be just great enough to draw a bubble of air between adjacent particles at the surface. This value is termed by Haines the "entry value." The first assumption appeared plausible, and was accepted by the writer; the second appeared untenable, but was not further discussed in view of the probability that the experimental values would have to be re-interpreted upon an entirely different basis. It will be shown in the present note that both assumptions must

be abandoned. The whole of the theoretical curve was thus either definitely erroneous, or conjectural upon an extremely slender basis.

When the values in the calculable portion of the curve were recalculated, it became evident that they were still in complete disagreement with Haines' experimental values, which, it could scarcely be doubted, must represent some real property of the soil aggregate examined, though possibly not its static stress under capillary forces. It is indeed almost impossible to think that this stress could characterise any portion of the aggregate in the neighbourhood of a rupture, for it is the stress under which the soil particles are in their original positions without mutual pressure, and any displacement in their positions must somewhat violently affect the film of moisture connecting them. On rupture, on the other hand, the particles are displaced so far that the connecting film is broken. There is thus little hope of obtaining measurements of the static stress by the method used by Haines, and the contradiction between his observations and the value calculated would be little to be wondered at, even if his material so far agreed in its properties with the theoretical ideal soil.

There is, however, one quantity intimately involved in the mechanics of the ideal soil which conforms in its general behaviour as water content is increased to the experimental values of Haines, namely the minimum work needed to cause rupture. This circumstance led me to suggest "that if these measurements can be equated to any of the mechanical properties of an ideal soil, it is work required to rupture the connecting moisture rather than the static stress exerted by it, that is the subject of measurement" (2, p. 497).

On this point and some others, Haines has returned to the subject<sup>(3)</sup> with the claim that "A decision as to other criticisms was left until more conclusive experimental evidence was forthcoming. This has now been obtained by direct measurement of the pressure deficiency, and the results fully confirm the original interpretation of the cohesive measurements."

The new experiments show that the pressure deficiency falls continuously with increasing water content, as must evidently be the case for a series of stable states; the change of pressure deficiency is, however, slow for water contents of 40-90 per cent. of the pore space, rapidly falling to zero as saturation is approached. These observations contain nothing to modify our previous conclusions. The value, however, at which the pressure deficiency passes its point of inflexion, at which point it may be said to be nearly stationary, or decreasing with minimum speed,

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seems in his new treatment to be identified by Haines with the "entry value" or pressure deficiency at which a bubble of air will be forced to pass between adjacent particles. It is not clear on what basis this identification is made. The observed point of inflexion occurs long before saturation. The experimental values are about  $6T/r$  (where  $T$  is the surface tension, and  $r$  the radius of the soil particles), while the theoretical entry value of Haines is, by rough but perhaps sufficient reasoning, assigned to the value  $12.8T/r$ . It is, of course, easy to ascribe such discrepancy to the differences which certainly exist between Haines' materials and the ideal soil which is the subject of calculation; the experimental particles are not of equal size, they are not, and cannot be, arranged in the arrangement postulated for the ideal soil; and, further, no evidence is adduced that the angle of contact of liquid with soil particle is at, or near to, zero. With these limitations in mind, these measurements cannot be taken either to disprove Haines' theory of a limiting pressure deficiency of 12.8, or, on the other hand, as showing that equilibrium subsequent to coalescence of the liquid rings is possible at higher pressure deficiencies than the value calculated at coalescence, which is 4.53; the latter is an interesting possibility on which Haines relies a good deal, but of which he gives no satisfactory proof. It may be noted that if the entry value is identified, not with the nearly stationary value of the ordinate, but with some point on the rapidly falling portion of the curves above 90 per cent. water content, the numerical discrepancy becomes more pronounced, while the arbitrariness of the identification is more surprising than ever. The measurements, which Haines has obtained of pressure deficiency, although contributing considerably to the problems chiefly considered in his later paper, do not therefore supply any conclusive evidence upon the problem of static stress originally attacked.

The pressure deficiency only enters into the latter question as a basis for justifying the supposed high stress value near to saturation. It was assumed by Haines (1), p. 532) that at saturation the static stress must equal the pressure deficiency; that this particular assumption is far from accurate may be proved by means of a general theorem applicable to all stages of water content, which would effectively dispose of the notion of a static stress rising to a maximum at saturation, even if it were admitted that the pressure deficiency were absolutely constant up to this point.

The theorem is as follows. The resultant force on a spherical particle of the fluid pressures and surface tensions to which it is exposed is that of a uniform pressure of amount  $p + 2T/r$  acting on the dry portions

of its surface, where  $p$  is the pressure deficiency of the liquid phase,  $T$  the interfacial tension, and  $r$  the radius of the sphere.

For proof, it is easy to see that the resultant of a pressure deficiency,  $p$ , over the wet portions of the sphere is equal to the resultant of a pressure excess,  $p$ , over the dry portions; the effect of the surface tension is probably most simply investigated by imagining the surface film to be continued by a membrane of uniform tension  $T$  passing over the dry portions of the sphere; then, such a membrane will evidently exactly balance the tensile force of the interface, and will itself be held in equilibrium by uniform pressure  $2T/r$  over the portion of the sphere which it covers. Consequently the resultant of the interfacial forces is equal to that of a uniform pressure  $2T/r$  over the dry areas.

The static stress may be at once investigated from the resultant forces; for these will be zero for particles in the middle of a soil mass, but will be inward for particles on the outer layers, so producing a static stress throughout the mass, the inward forces being ascribable to the excess of dry area upon the outer surfaces of the outmost layer of particles. If, therefore,  $A$  is the projection of the dry areas on the outmost layer upon a unit area of surface, and  $\alpha$  the projection of the corresponding areas of particles within the mass, the static stress may be written as

$$(p + 2T/r)(A - \alpha).$$

One can now see how slender are the opportunities for the stress to increase in any portion of the range, and that it cannot increase steadily up to a large maximum at saturation. For  $2T/r$  is constant for all water contents, and  $p$  is a quantity which decreases, or in a limiting case remains stationary, with increasing water content; the first factor therefore never increases but usually decreases. Nor can the dry area at the surface be expected to increase with increasing water content, and the only chance of the second factor increasing in any part of the range is that in such a part  $\alpha$  should decrease more rapidly than  $A$ . The possibilities for this are very limited, for  $\alpha$  is much the smaller quantity, and we can at all stages assert that the stress is less than  $(p + 2T/r)A$ , a quantity which cannot increase at any stage.

If, for close packing, we evaluate  $A$  and  $\alpha$  at coalescence, we find

$$A = \frac{3}{4}, \quad A - \alpha = \frac{\pi}{4\sqrt{2}} = .5554, \quad \alpha = .1946.$$

There is thus no possibility of identifying the stress with the pressure deficiency,  $p$ , especially at saturation, when the area  $A$  must tend to zero.

In view of the above theorem and its consequences, it will be un-

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necessary to go into several points in Haines' last paper which indicate that he has misunderstood the criticisms of his original treatment of static stress.

A very simple reason may be given for rejecting the "entry value," not necessarily from the interpretation of Haines' experiments on pressure deficiency, which may be largely influenced by this value, but certainly from the series of pressures at which the soil water is in physical equilibrium with the surrounding atmosphere. For the process by which, according to Haines, air enters or leaves the cells of the soil structure involves the dissipation of energy; a film of liquid is supposed to be pressed upon by the intruding air until it is ruptured, the air then "blows through" the opening so formed; the energy which must be dissipated before mechanical equilibrium can be again established gives rise to a series of jumps, sufficiently sharp even to have visibly affected the manometer in Haines' experiments (3), p. 273). It will hardly be denied, therefore, that such a rupture involves irreversible processes by which energy is dissipated; and it follows that the corresponding adjustments by reversible changes will take place at a lower value of the pressure deficiency. The agency for such reversible changes of air content being, of course, furnished by the air dissolved in the soil moisture.

### SUMMARY.

The new observations of Haines on the pressure deficiency of liquid in a soil-like aggregate confirm the theoretical deduction that the pressure deficiency falls off with increasing water content, but do not justify his belief in a high static stress as saturation is approached.

A theorem is established connecting the static stress at any water content with the pressure deficiency and the dry area of the surface. This, together with general considerations of the energy conditions of physical equilibria, appears to dispose of the two assumptions from which the high values for the cohesive stress at saturation have been deduced.

### REFERENCES.

- (1) HAINES, W. B. (1925). "Studies in the physical properties of soils. II. A note on the cohesion developed by capillary forces in an ideal soil." *J. Agric. Sci.* **15**, 529-535.
- (2) FISHER, R. A. (1926). "On the capillary forces in an ideal soil." *Ibid.* **16**, 492-503.
- (3) HAINES, W. B. (1927). "Studies in the physical properties of soils. IV. A further contribution to the theory of capillary phenomena in the soil." *Ibid.* **17**, 264-290.

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## STUDIES IN THE PHYSICAL PROPERTIES OF SOIL.

### V. THE HYSTERESIS EFFECT IN CAPILLARY PROPERTIES, AND THE MODES OF MOISTURE DISTRIBUTION ASSOCIATED THEREWITH.

By WILLIAM B. HAINES.

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(With Six Text-figures.)

#### INTRODUCTION.

IN two former papers (1, 2) the writer has dealt with the capillary properties of moist soil from both a theoretical and experimental standpoint. The first paper was followed by R. A. Fisher(3) with the presentation of certain emendations and criticisms, together with an elaborate treatment of a special part of the ideal case. Some of these criticisms were incidentally examined in my second paper in the course of a more extended treatment supported by fresh experimental results. Fisher returns to these points in a recent communication(4), and, among other arguments, makes the claim that a new theorem regarding stress must be taken as leading to a final rejection of certain of my views. It appears that this theorem is an elaboration of my first conception, and, while it gives precision where my own statement was crude, it is, nevertheless, in essential agreement with those first descriptions. In the present paper it is hoped to resolve the differences of opinion by the introduction of a fresh consideration which throws former arguments into a better perspective by showing where their basis was incomplete. Briefly, the new work has shown that two distinct cases must be considered, one for rising moisture and one for falling moisture. Confusion has been introduced and unfounded criticisms offered by the tacit assumption that moisture changes would form a reversible series, so that arguments applied to the features of one case have been used to exclude the possibility of the other. The changes in capillary suction which accompany cycles of change in moisture are shown in the experimental part of this paper to move round hysteresis loops rather than to and fro on a reversible curve. Neither theoretical discussions nor experimental studies have hitherto sufficiently emphasised this important distinction, and it constitutes a valuable standpoint for further work.

As an introduction the following simple illustration may be given of the essential basis upon which the hysteresis effect rests. Consider a vertical capillary tube having regular variations in its bore (radius  $r$ ), dipping into a liquid by which it is wetted (surface tension  $T$ ). In Fig. 1 let the sinuous line represent the characteristic of this tube, showing for any height above the free water surface the value of  $2T/r$ , which is the pressure deficiency shown by a liquid-air meniscus in the tube at the given place. The straight line  $OS$  represents the reduction in hydrostatic pressure with height for a liquid column in the tube. The points of intersection,  $R$ ,  $X$  and  $S$ , where the abscissae of the two curves are equal, give the positions at which a meniscus can be in equilibrium with the column of liquid beneath it. The position  $X$  is unstable however. A slight displacement of the meniscus above  $X$  causes its pressure deficiency to outbalance the pull of the liquid column, and it moves up to position  $S$ . Thus an addition of water results in a rise of pressure deficiency.

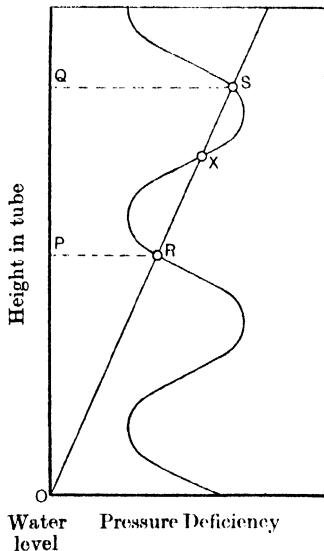


Fig 1.

Similarly a displacement in the opposite direction causes a fall of the meniscus to  $R$ . Such *per saltum* movements have great importance in the analogous case of movement of the water boundary in the soil, when the moisture content is raised or lowered.  $R$  represents the position taken up for capillary rise of the column, while  $S$  is the position reached by a falling or draining column. By raising or lowering the tube the extreme values of the two heights will correspond to the extreme values in the fluctuation of bore.

Inasmuch as the pore space of soil has a cellular character, or a variable cross section, its capillary properties partake of the nature of those of the tube described. Except for limited ranges where reversible conditions may hold, the mode of moisture distribution in soil does not give reversible conditions but leads to two main values of capillary pull. The case of falling moisture tends to be governed by a higher value of pressure deficiency as determined by the narrower section of the pores, while conditions of wetting or increasing moisture tend to be governed by a lower value depending upon the wider sections of the pores. In other words, it is found that a granular system, of which soil is typical, will in

general offer a greater capillary pull against the extraction of water from its pores than it can engender when absorbing water into them. The distinction was clearly made in a former paper (2) when comparing capillary rise with drainage, but the full implications were not then developed.

#### THEORETICAL.

The theory of the subject has dealt with an assemblage of uniform spheres as the ideal soil. Two regular types of packing have been discussed, cubical and hexagonal, of which the latter will suffice for the recapitulated considerations which follow. The condition which determines the mode of distribution of any given amount of soil moisture is that the curvature at all points of the air-water interface shall be the same. This curvature is conveniently expressed in terms of the pressure deficiency or suction to which it gives rise in the soil moisture, and the natural unit for its measurement is the ratio  $T/r$  ( $T$  = surface tension,  $r$  = radius of particles). It is assumed that the effects of gravity may be neglected and that the angle of contact between liquid surface and solid particle is zero.

The nomenclature of Versluys(5) will be adopted for the types of distribution which arise. The lowest moisture range gives rise to isolated discs of water around the points of contact between particles, which is called the *pendular* case. This is the case which has proved amenable to full mathematical treatment. Its limit is reached when the discs are large enough to come into contact at their edges, which happens for a water content corresponding to 24 per cent. saturation of the pore space. But over a large part of that range of moisture the pendular form of distribution is not unique. For all higher moisture values the water must form a continuous body. Here two general types of distribution are distinguishable. In one, the *funicular* case, the discs of the pendular case become joined by fusion at their points of nearest approach, forming a meshwork with air still occupying the wider spaces of the pores. In the other, the *capillary* case, the water boundary which is considered encloses a saturated region of soil.

The cellular character of the pore space in the ideal soil has already been described in detail (2). It will be sufficient for the present purpose to recall that the dimensions are such that a hemispherical meniscus at the widest section (cuboidal cell) has a pressure deficiency of  $6.9 T/r$  while one at the narrowest section (channel joining two cells) has the value  $12.9 T/r$ . For the pendular case very small discs have a very high pressure deficiency which falls as the discs grow in size, reaching the lower limit of  $4.5 T/r$  at the point where they are large enough for coalescence to

begin. This value is relatively low because of the anticlastic form of the water surface in this case. It may be likened to the deeply hollowed rim of a motor wheel as it is prepared to take the pneumatic tyre.

Changes of moisture within the pendular range may be made reversibly and with entirely symmetrical distribution through equilibrium with a soil atmosphere of changing relative humidity. For the other types of distribution with a continuous film, equilibrium is adjusted by direct flow of water, and changes are best considered as film movements or changes in curvature following direct increments or decrements of water. In the case of any soil air becoming trapped the means of attaining equilibrium becomes solution of air in the soil water. The distribution is not always symmetrical for higher moistures.

The total surface area of the interface between the soil moisture and soil air plainly passes through a maximum somewhere between zero moisture and saturation. On one side of this maximum the water surface increases with moisture, but on the other side it decreases with rising moisture. This leads one at the outset to doubt whether the entire range of changes could be conducted as a smooth reversible series of stable states.

It will be best to consider first in more detail the stable film forms which can exist, as determined by the geometry of the pore space, before proceeding to discuss the manner in which they may be associated for any given moisture content. The most simple case is the saturated or capillary one represented in Fig. 2 in a purely diagrammatic fashion. At (a) the fully saturated case is shown with a plane air-water boundary. For a very small water decrement the film is drawn into the surface pores of the soil as in (b). These pores have waist-like constrictions and open into wider cells beyond. The cells are of two kinds, the larger type cuboidal and the other tetrahedral. As the meniscii in the external pores advance inwards under the extraction of moisture from the soil, the pressure deficiency rises until the narrowest point is reached, when it has a value in the neighbourhood of  $12 T/r$ . This might be called the liminal case of saturation. At some pore which is minutely wider than others the meniscus next passes the unstable point and expands abruptly into the cell beyond ((c) in Fig. 2). The displaced water redistributes itself with a slight general fall in the pressure deficiency. The shape of the film in the evacuated cell is made up of meniscii, one at each corner (except the one of entry), ready to penetrate farther into the soil by a similar leap. These meniscii are joined round the cheeks of the enclosing particles by films which are really broken-up portions of the pendular discs. This is the

funicular type of distribution. For further decrements in moisture the process of evacuation extends cell by cell through the soil. During the extraction of each quantum of water represented by a cell's contents the pressure deficiency rises gradually by a small amount (for a series of reversible changes), and then falls suddenly again (irreversible change). By repetitions of this process a large portion of the water is lost at a pressure deficiency oscillating slightly about the value at which entry first took place (entry value  $12 T/r$ ). The change is a simple movement, in quantum jumps, of a boundary between a saturated region of the soil and one having a funicular distribution.

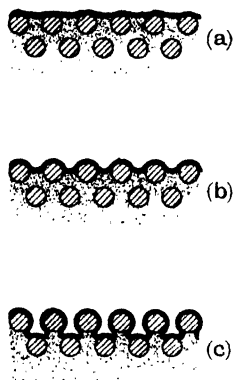


Fig. 2.

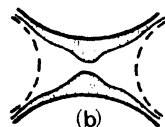
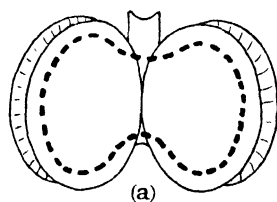


Fig. 3.

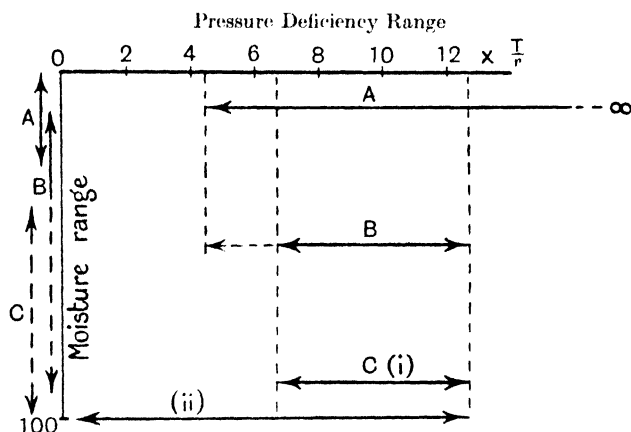
Consider now a reversal of the direction of moisture change at the stage represented by (c) Fig. 2. As the moisture increases and the pressure deficiency decreases the water film in each cell sags towards the centre, until in one cell (whichever is the smallest) the bubble detaches itself from the walls and collapses by evacuating the air through the external open pore. The point of instability for this is in the region of  $6.9 T/r$ , the pressure deficiency of a bubble in the large type of cell. The entry remains "open" up to that point because the pressure would have to fall much lower to  $4.5 T/r$  before closure takes place at a waist. The smaller type of cell will fill at a higher pressure deficiency, but they are always separated from one another by larger cells; hence they will fill in association with their larger neighbours in a manner which can be varied according to the suppositions made. Thus we see that a reversed movement of the boundary

round a saturated region takes place in a manner similar to its complementary case, that is by jumps, but at a lower pressure deficiency. Some confusion entered my former paper (2), p. 273) by mistakenly identifying this value with the entry value, *i.e.* by not correctly separating the cases of rising and falling moisture.

A special feature relating to the funicular and pendular types of distribution is the existence of alternative positions which the film may take up in the waist-like connecting channel between two cells. This may be full of water with a meniscus at each end, or it may be open with an air-passage through. The receding film in the falling moisture case will open some passages, but leave others closed. Thus a cuboidal cell has eight corners, only two of which need to be opened by the transit of the water front, one for entry and one for exit. In Fig. 3 a diagrammatical presentation of a unit of the funicular case is given. At the limit of the pendular stage we have three "wheel-rim" surfaces set together at  $120^\circ$  with edges just in contact, forming an open hour-glass channel between them. In the full case each disc makes several such contacts with others round its periphery. At (b) is shown a diagram of a longitudinal section through the waist. As the discs close together by the addition of water a sudden closure takes place and the new surface is indicated in broken line in both (a) and (b). This closure is approached at a value of pressure deficiency in the neighbourhood of  $4.5 T/r$ . As we have seen, when the moisture falls, the passage is only opened again for a pressure deficiency equal to the entry value. Thus full merging of the discs takes place for rising moisture at  $4.5 T/r$  or lower, while their breaking up for falling moisture takes place at  $12 T/r$ , which will give rise to discs of comparatively small size. These therefore are the two limits of pressure deficiency between which there exists the alternative of a waist being either open or closed, although it will be shown that the case for closed waists has only a limited possibility below the value  $6.9 T/r$ . With sufficient waists closed to ensure moisture continuity we have the funicular case, within which changes are reversible and run approximately parallel to the values already given for the pendular case by Fisher(3).

The above facts are condensed in Fig. 4, which shows the range of pressure deficiency for each type of distribution. It is plain from this that all the various forms could be in equilibrium together within certain limits of pressure deficiency. There is by no means a close association between a given moisture content and a certain distribution, nor is the distribution usually uniform throughout the soil. The moisture ranges have been put in to show how they overlap, although the value 24 per cent.

saturation for the limit of the pendular case is the only one yet accurately determined. The overlapping between the moisture ranges of *A* and *B* is a genuine alternative distribution, but that between *B* and *C* is shown in broken line because part of the moisture is distributed in one form and part in another according to the amount present. The possibility of a partial development of the funicular case at pressure deficiencies less than  $6.9 T/r$  is also shown by a broken line. Various cases of distribution can be arrived at according to the sequence of changes leading up to them. Over the greater part of the moisture range two regions exist side by side, one saturated and the other having a low moisture. This is modified when



*A*, Low moisture range. Pendular case up to complete coalescence (closure of waists).

*B*, Intermediate moisture range. Funicular case (after closure of waists).

*C*, High moisture range. Capillary case: (i) local saturation only; (ii) complete saturation.

Fig. 4. Scheme of various modes of moisture distribution and the ranges they cover.

a non-uniform soil is being considered. In an irregular medium the pore sizes vary, and the changes discussed tend to follow in sequence through groups of pores graded according to size. At any stage cells smaller than a given size will tend to contain water and cells larger than this to contain air.

One outstanding point remains, namely, to reconcile the value  $4.5 T/r$  at the end of the pendular stage with the subsequent value  $6.9 T/r$  when the funicular stage is giving place to saturation. In my earliest remarks (a), p. 531) the view was taken that the end of the pendular stage would be an unstable case followed by a rise in suction. It is plain that the point is approached at which further addition of water leads to decrease in surface area. For first the waists will close and then the cells. The

mechanism of the transition is not difficult to follow. At the end of the pendular stage the film shape in a unit cell can be imagined as a cuboidal (or tetrahedral) bubble rounded at the edges and drawn out at each corner to a cone-like extension, where it joins the neighbouring units in the hour-glass waists already depicted. These cones supply the outward tension which stabilises the natural inward tendency of the bubble to collapse. Closure of a waist corresponds to the collapse of the cone, and disappearance therefore of the compensating tension. This process will ultimately upset the stability of the bubble, which collapses, filling the cell with water and starting a region of saturation. To determine how many corners could be closed before instability is reached for each kind of cell would provide an interesting subsidiary problem. It would also illustrate the different ways in which rising moisture could be carried out by suitable choice of the order in which the waists are closed. Former arguments (2) were complicated unnecessarily by supposing that air would be trapped, but the closing of the waists in sequence could be conducted so as to avoid this. The important conclusion, which seems to the writer unavoidable, is that at some point the pressure deficiency will rise to  $6.9 T/r$  for the completion of the range. The system is brought to a certain point at a pressure deficiency of  $4.5 T/r$  (or rather lower) for which as many waists have been closed as is compatible with stability. Then the next closure of a waist is a trigger action which causes first one cell and then the next to close (compare a row of skittles). In this way the saturated zone extends, and a new equilibrium is not arrived at until enough water has been withdrawn from the other regions of the soil to raise the pressure deficiency to  $6.9 T/r$ . The rise depends upon this general redistribution. The new case has a greatly reduced surface area, which meets difficulties raised from energy considerations. The readjustment gives the normal case for rising moisture over the higher range. Any alternative mechanism by which the last stages could be conducted at a pressure deficiency as low as  $4.5 T/r$  would have to depend upon limiting the closure at the waists, if this would confine the unstable region to the zone in contact with the saturated water front. The point is rather an academic one, and only derives importance from the contrast between the theoretical approach from the coalescence of pendular discs which starts with the waists open, and the practical approach for cycles of moisture change which finds most of the waists remain closed.

Fisher has thrown doubt on this rise in the pressure deficiency above  $4.5 T/r$ . Originally his considerations applied to "the series of states of the soil-water system in equilibrium with successive values of relative

humidity" as affording "a definite basis of comparison over the whole range from absolute dryness up to saturation" (3, p. 495). Such a series simply excludes a rise in pressure deficiency (synonymous with a fall in relative humidity) by definition, and is not necessarily valid for the criticism of a series defined by rising moisture content and passing through points of film instability. The relative humidity series passes from the unstable point directly to the case of final saturation for its next equilibrium position. For such a limited series Fisher's contentions may readily be conceded, but it may be remarked that the series gives undue prominence to the pendular (calculated) case while omitting cases of much more practical importance.

The points mainly contested by Fisher have concerned the stress or cohesion developed in the ideal soil. In my first approach to the subject I had considered the case of a saturated soil mass entirely enveloped by an inward-pressing water film. This led to the assumption that the cohesion for this case would equal the pressure deficiency, and a value between  $6.9\ T/r$  and  $12.9\ T/r$  was assigned to it. Fisher elaborates this conception of cohesion as produced by the inward thrust of the external layer of particles in the following theorem. If, for any layer of particles, the dry areas be divided into two groups, one having an outward aspect relative to the soil mass and total projection  $A$  per unit area and the other having an inward aspect and projection  $\alpha$ , then the pressure exerted inwards depends upon the excess of  $A$  over  $\alpha$  and is written

$$\text{stress} = (p + 2T/r)(A - \alpha),$$

where  $p$  is the pressure deficiency in the moisture. Applying this formula there appears no essential conflict with previous conclusions. For the approximate first assumption for the saturated case we see that the term  $2\ T/r$  was overlooked, while  $A$  was given the value unity; whence stress =  $p$  since  $\alpha$  is zero. In the more exact treatment for the liminal case of saturation we have  $p = 12\ T/r$ ,  $A = 0.75$ , and  $\alpha = 0$ . This case gives the maximum value of the formula, about  $10\ T/r$ , so that my estimate of a value lying between  $6.9\ T/r$  and  $12.9\ T/r$  cannot be regarded as deceptive. Nor was it deceptive to associate maximum stress with saturation. It may be noted in passing that it was the experimental agreement between the values of stress and of pressure deficiency at saturation in a sample of silt which led to the reopening of the argument. Both experimental arrangements had given the falling moisture case. The indication now is that this agreement was closer than was justified by the simple theory. The value found was  $6\ T/r$  for both measurements, and it is seen that the two deviations of the rough treatment from the exact one (supposing

direct application of the ideal formula to the real case) just happen to cancel for such a value.

Tracing the changes in cohesion as the moisture is decreased it is plain that  $\alpha$  suddenly assumes positive values at the region of entry, without much change in  $A$  or  $p$ , so that cohesion falls. It seems that now there are two regions of different stress; that having the funicular type of distribution and a stress  $(p + 2 T/r) (A - \alpha_1)$  given by the external layer of particles, while the saturated region has an additional  $(p + 2 T/r) \alpha_2$ , for the layer at the saturated water front. Thus the saturated region remains under the maximum stress. When the direction of moisture change is reversed the stress will be smaller, but similar conditions will hold. As the water front extends and obliterates  $\alpha$  by filling the cells, the saturated region so formed gains an increased stress. The possibility of stress increasing with moisture for the later range seems clearly established.

Fisher comes to entirely contrary conclusions as to the application of the above formula, considering that it "effectively disposes of the notion of static stress rising to a maximum at saturation." Such reasoning must obviously depend upon the moisture distribution and as this is not described the argument lacks in definiteness. Although the formula is so well adapted for the exploration of the more important high moisture stages, Fisher contents himself with showing that it verifies the value already established for the end of the pendular stage. No sufficient guidance is given for an estimate of  $A$ ,  $p$  or  $\alpha$  over the range in question, although a general consideration of their changes is shown to lead to the conclusion that, if stress increases with moisture, then  $\alpha$  must decrease more rapidly than  $A$ . Regarding this key condition it is simply stated that it has very limited possibilities. It has been shown, however, to be possible for the case discussed above. It seems that the changes in mind are smooth and reversible ones. If such a series can be theoretically established it may conform to Fisher's conclusions. But it must be entirely different from the changes here described, and shown in the next section to be reproducible experimentally. Since the irreversible series fall into line with experimental observations, they must be regarded as the ones which are important for consideration in practical problems relating to soil. In both his papers it is clear that Fisher, while contributing much to precision, has been in error in casting doubt upon the idea that high stress and high suction can arise for a saturated condition in the practical case.

## EXPERIMENTAL.

In order to give the whole subject better objective illustration some simple experiments will be described in which the cycle of moisture changes was investigated under controlled conditions of pressure deficiency. The measurements bearing most closely on the ideal case were made by means of paraffin oil and phosphorbronze bearing-balls of  $\frac{3}{32}$  inch diameter. These are large enough to arrange individually in the correct packing, but small enough to give measurable capillary effects. Time was not available to arrange large numbers for examination in bulk, but measurements were obtained for the behaviour in single cells in the following manner. A lead tube was taken for one arm of a U-tube and the top was shaped to take a porous plug of exactly the dimensions to form a plane surface on which a basic layer of particles in hexagonal packing could be laid. These were held in position by turning the edge of the tube slightly in the same manner as in setting a gem. Further layers could then be built up. The U-tube was completed with rubber and an adjustable second arm. This was then filled with oil, and the behaviour of the oil film between the particles examined through a lens while adjustments of pressure deficiency were made by raising or lowering the second arm. All entries between particles except those in the upper layer could easily be closed by placing sand or glass particles of appropriate size against them. This was desirable for studying strictly local changes. Measurements of head were made with a travelling microscope, while the surface tension was checked in all cases by means of the capillary tube method and by du Noüy's apparatus.

Under saturated conditions and for increasing suction the oil film could easily be seen making its abrupt entry into the first layer of cells. This value of pressure deficiency was between 11.3 and 11.5  $T/r$ . For the return by diminishing values of suction the filling of a cell was equally abrupt, and could be demonstrated to take place first in those of tetrahedral form. The pressure deficiency value for this return again substantiated the theoretical values already discussed. If the receding film for preparation were dragged far, so that the pendular mode was mainly induced, the larger cells filled for the return at the value 4.3  $T/r$ . This verifies the value for the coalescence of the pendular discs and demonstrates the subsequent instability in the immediate closing of the cell. The attainment of the low value simply depended upon the inability of isolated discs to adjust themselves as the pressure deficiency decreased, so that the saturated front as it advanced affected only those discs in actual contact

with it, the remaining region still having a high pressure deficiency round 11. In that case part of the contour of a cell remains unaffected until collapse. If, however, a single cell was dealt with, round which the oil continuity was such as to allow the whole film to sag naturally as the pressure deficiency fell, the collapse was at the value 6.7  $T/r$ . The agreement with theory is so close that these higher values can never again be called in question.

A series of measurements of greater practical importance was made upon the material known as glistening dew. This is a tinsel consisting of minute glass spheres of very considerable uniformity, and it may be taken as closely simulating the ideal soil in all respects except closeness and regularity of packing. Green and Ampt (6) have described it in their own studies of soil permeability. The mean particle radius of the sample used was 0.019 cm. The natural packing, which could be altered slightly by the degree of shaking, gave a value of pore space between 36 and 37 per cent. or half-way between the ideal cases of close and open packing. The material was held in a Büchner funnel over a carefully sealed filter paper. This formed the terminal of one arm of a U-tube whose other arm was formed by a burette with double-bored stopcock (Fig. 5). The whole apparatus was filled with liquid and could easily be manipulated to apply changing suction to the material in small steps by adjusting the height in the burette. The resulting increment or decrement of moisture at each step after equilibrium was reached was read off on the scale. Reversibility could be tested for each section of the curves so obtained. The datum level for pressure was taken at the surface of the glistening dew. The whole mass had a depth of 2 cm. (and diameter of 9 cm.) and the results are therefore affected by this difference in gravitational head between the top and bottom of the material.

The irregular packing gives rise to a variable pore size, which is in the main wider than that for the ideal case and thus gives lower values of pressure deficiency. From the manner in which the water-air interface penetrates for falling moisture, it is apparent that the entry will be effected *last* into those places where the closest packing exists, that is, to such places as reproduce the ideal case. Therefore the ideal value for

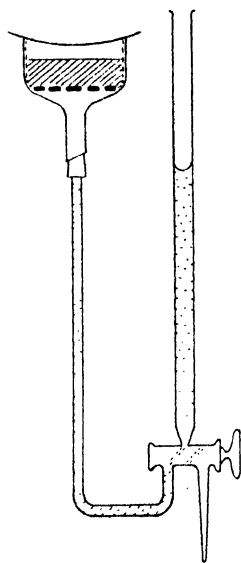


Fig. 5.

entry may be expected to be shown at the end of the funicular stage. Conversely, for *rising* moisture, closure will take place *first* at the same places, and the ideal value will correspond to that point at which closure (and the consequent onset of irreversibility) can first be demonstrated.

A complete series of curves is shown in Fig. 6 with arrows to indicate the direction of the moisture change. Where the curve represents changes which are reversible it is drawn in double line. The nature of the experiment, of course, limits the findings to the case in which film continuity exists. The convenient origin for plotting was taken as 100 per cent. saturation and zero pressure deficiency. A glance at the figure shows the marked distinction between the falling and rising moisture cases, and the predominance of irreversible conditions. It is clear that there is no unique association between particular values of pressure and moisture. The material can be brought to the state represented by any point within the hysteresis loop by suitable cycles or raising and falling moisture. Over those stages where the curve is nearly vertical the moisture is not uniformly distributed throughout. As in the ideal case, a region of low moisture exists in equilibrium with a saturated one, and moisture changes go on by encroachment of one region on the other by movements of the boundary film. The existence of a gravitational gradient of pressure in the glistening dew determines that this boundary movement keeps to a horizontal plane.

Drying conditions usually predominate over wetting whether in the laboratory or field, so that the falling moisture curve *OABCDE* is the one of main interest. It is the same as that previously obtained by means of the manometer and porous pot (2). From *O* to *A* the pressure deficiency rises with only a small moisture movement confined to the rugosities of the surface layer. The change has already been depicted in Fig. 2, and is accompanied by a change in appearance from shiny to matt. At *A* the entry value for open packing is reached, and the first penetration of the film through the surface layer of particles takes place at isolated points. These points of penetration are plainly visible. As entry to a cell is effected the  $\alpha$  dry areas (of the theoretical treatment) are formed inside, which greatly increases the internal reflection of the glass particles concerned. Entry is therefore accompanied by a sudden scintillation of the particles at the particular spot. General entry does not take place until the average value 6.1 is reached, indicated by the sharp bend in the curve at *B*. About 70 per cent. of the water is thereafter lost at this value. The portion of the curve from *B* to *C* would be quite vertical if corrected for the depth of the glistening dew (gravitational head).

An interesting indication of the effect of packing was obtained in one curve which showed a hump due to lower values of pressure deficiency obtaining after entry through the surface layer of particles. This was due to the extra close packing of the surface layer resulting from the greater freedom of the surface particles to roll about and adjust themselves.

Along the curve *CDE* the water loss is being supplied by evacuation of cells having smaller entries than the average and also by reduction of moisture under the funicular mode or film form. At *E* the funicular stage ends, the abrupt break being clearly indicated in the curve. No further water is removed by increases in suction. The value of moisture at *E* is 8 per cent. saturation, while the pressure deficiency should be that of the entry value of the ideal case as already indicated. Since the final break must occur at the junction between the glistening dew and the filter paper on which it rests, the value of  $11.2 T/r$  on the curve at *E* requires to be corrected for this level. This gives the value 10.6 to compare with the value 12 given in the theoretical section. The suction value at *E* was the same for all repetitions of the curves, although there was some slight variation in other regards due to slight differences of packing obtained on different occasions. It is seen that the strict pendular case is confined under these conditions to the lower third of the moisture range which can be assigned to it in the theoretical treatment.

The curve for the rising moisture case is best followed by reversing at *D*, just before the break up of the film continuity. For the first part the returning curve runs parallel to the ideal curve for the pendular case (shown in broken line), since in the main the film forms correspond; that is, we have portions of rings joined together by those entries which have remained closed by reason of their tightness. This part of the curve is reversible. At *F* the curve bends away from this parallelism owing to the beginning of closure at the cells of tightest packing. This is also demonstrated by the fact that at *F* the curve begins to be irreversible. The pressure deficiency value at *F*, corrected for the depth of glistening dew, is 6.4, which is very near to the theoretical value for returning moisture. General closure for the average sized cell comes later at *G* for a pressure deficiency value of 4.1. It is interesting to notice the more angular nature of the change in the curve at *B* than at *G*. This is due to the more extensive air-water film in one case than in the other; so that in one case (*G*) cells of any one size throughout the mass have an almost equal opportunity to fill with water at their appropriate value of pressure deficiency, while in the other case (*B*) only those along the particular bounding water front have opportunity of evacuating water.

Saturation is practically complete at  $H$ . The unfilled volume of about 12 per cent. represented by  $KO$  is the air trapped in the process of closure, which tends to happen for abnormally big cells.

The experiments were repeated with paraffin oil instead of water, and exactly similar curves obtained. In this case the stable values were 5.9  $T/r$  for falling "moisture" and 3.9  $T/r$  for rising "moisture." The latter value is in perfect agreement with the mean value derived by Hackett(7) from measurements of the capillary rise of oil in sands.

A number of intermediate curves are shown illustrating the effect of reversal of the direction of moisture change at various stages. If the funicular stage is carried to its limit, the returning curve is via point  $L$ . Owing to the break up of film continuity and absence of the usual mechanism of adjustment, the form of the curve in such a case depends very much more than usually on the time allowed for equilibrium to be reached. The inside curve at  $L$  was obtained for very long periods (24 hours) between each reading.

The curve drawn in dotted line was obtained for a sample of sand with rounded grains. The much greater variation of grain size in this case gives rise to a finer pore space by adaptation in packing, and hence the suction values are higher. But the character of the two curves is unaltered.

As previously indicated (2), pp. 275, 284) these curves may be viewed as showing the equilibrium distribution of moisture in a sand column above a free water table, if the suction values are first transformed into the heights which give the same gravity head. The right-hand curve is the case for drainage and the left-hand for capillary rise. The distinction needs great emphasis, since a great many studies of moisture distribution above a water table have been made without taking it into consideration. Indeed, it has generally been assumed that there is a single series of reversible changes.

The work spent in removing moisture is  $\int p dv$ , or the area between the curve and the moisture axis. In the case of glistening dew and water the curve from  $O$  to  $E$  represented 42 c.c. of water removed, and the expenditure of  $1.02 \times 10^6$  ergs. This is of the same order as the surface energy of a water film equivalent in area to the particle surface. Both quantities reckoned per unit volume of soil vary as the reciprocal of  $r$  and therefore change in the same way with the texture of the soil. The area of any hysteresis loop is the energy wasted irreversibly.

Since writing the above an important contribution has been made to the subject by Hackett and Strettan(8). Their work elucidates the transition from close packing to the "common" packing naturally

assumed, so forming part of the essential bridge by which the theoretical case can be applied to real soils. Their treatment applies to the narrower pore sections which govern the falling moisture case. Their experiments are closely parallel to those reported here. Using steel balls and paraffin oil, the entry value found for close packing was the same as my own, namely  $11.4 T/r$ . The manner in which it falls off with the angle of packing is very clearly worked out. The value for glistening dew is a good deal lower than mine at  $4.75 T/r$ , but the difference is entirely explicable on comparing the two methods of measurement. While my own gives the value for the main group among the pore sizes, that of Hackett and Strettan gives the value for the largest pores. They cause a "saturated water front" to descend by drainage through a column of glistening dew resting upon wire gauze, and find the value for which it breaks through at the bottom. This takes place at the largest aperture available, as the authors clearly realise. Since, however, the stated aim is to infer the height of capillary rise in soil, the value  $4.75$  cannot be directly applied, and, compared with my direct measurement of rise, it must be regarded as a high rather than a low estimate for this. Actually the wire gauze itself forms the lowest stratum of apertures, and it would form with the bottom layer of particles a series of pores of different shapes to those which characterise the mass. Although an error due to such a cause would hardly escape the experimenter's attention, especially as the film movements were visible, yet the point is one requiring mention. In my own arrangement the boundary was the extreme case of such minute pores (of a filter paper) as to form a fixed barrier for the range of values being explored. In the other arrangement the supporting gauze must go to the opposite extreme and provide free passage through apertures larger than those which are required to be measured.

As regards stress between particles, certain observations can be made on the way in which this affects the friction between them. The glistening dew undergoes a most noticeable increase in firmness or rigidity while the suction is rising to the entry value, that is, between  $O$  and  $B$  in Fig. 6—very much greater than any subsequent change. A measurement was made of the depth of penetration of a loaded inverted cone into the glistening dew at different stages. The stress as so indicated was at stage  $B$  (liminal case) some ten or twelve times that at  $O$  (full flooding), which clearly proves the marked difference which can be produced by a very minute moisture change. The fall in stress subsequent to penetration of air was just indicated, but was too small to be definitely measured by the rough apparatus available.

The changes in appearance and in rigidity between *O* and *B* must be familiar to everyone in the behaviour of sea-shore sand under the foot when walking near a receding tide. The conditions then exactly reproduce

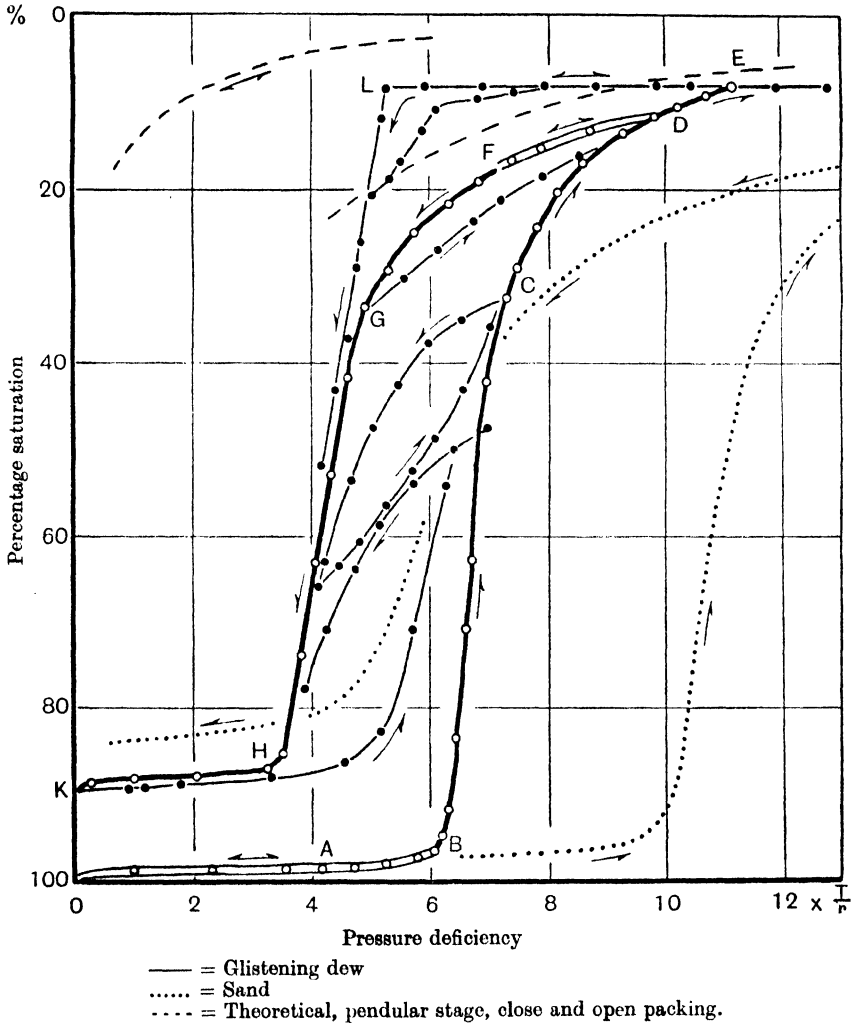


Fig. 6.

those in the above experiments. The sand is saturated and under a small pressure deficiency. The additional property which comes into play is the Osborne Reynolds effect, namely, the anomalous dilatation of a closely packed granular medium when subjected to an external force. Pressure such as is given by the foot causes an expansion in packing and an increase

of void volume in the neighbourhood. This produces conditions equivalent to a movement along the curve *OB* in the direction of *B*, that is, to an increase in suction and in rigidity accompanied by a more matt appearance of the surface. If the pressure is maintained, a flow of water takes place toward the compressed region until the hydrostatic pressure is equalised. The imprint then left on removing the foot glistens (floods) for a moment, since the excess of water is released as the former packing is resumed. If the surface of the glistening dew be pressed by the finger at a similar stage in the above experimental arrangement, the Osborne Reynolds effect is demonstrated by a *fall* in the water level in the burette, showing the increase in pore space and in suction.

The criticisms raised by Fisher<sup>(4)</sup> can now be seen in their true perspective, and the matter may be closed with the following few remarks. The calculation of stress or of work for the pendular case in the ideal soil I cannot regard as having direct application to my measurements made upon silt, since I have stated my disbelief that this distribution was ever actually attained (11, p. 535). The experimental cohesion curve given, showing a maximum at saturation and a falling curve thereafter, plainly has the values and follows the course to be expected from the descriptions of this paper, bearing in mind that in the experiment the material was always prepared under drying conditions, so giving the falling moisture series.

Independent evidence of the increase of cohesion with moisture is afforded in the recent work of De Witt and Brown<sup>(9)</sup>. These workers employed a much more accurate technique for determining the breaking strength of moulding sand. In the case where water was the only bond they report rising strength with rising moisture, while a rough calculation of particle size based upon the screening figures reduces the highest strength reported to the value  $6 T/r$ , which was the value obtained by the writer.

Fisher's reference (4), p. 410) to my demonstration of the micro-oscillations in the pressure deficiency associated with the quantum movements entirely confuses their significance. So far from being a series of oscillations after rupture of a film before a new mechanical equilibrium is established, they correspond individually to the invasion of a cell. My description showed that the rising half of each oscillation represents a reversible series of equilibrium values by which the limiting value of pressure deficiency is approached. A sudden fall completes the oscillation as entry to a cell is effected. The free oscillations consequent on this last adjustment are the ones referred to by Fisher, but they are

so highly damped as to escape attention in the apparatus which was described.

Fisher also expresses some confusion as to the point on the experimental curves which was identified with the entry value. The point of inflexion mentioned by Fisher, while useful as indicating the entry value for the main group of pore size, was not explicitly referred to. By definition the entry value was associated with saturation, and a comparison between the real and the ideal curves was relied on to make it plain that the usual convention was followed by which a sharp bend in an experimental curve is treated as an approximation to the intersection of two straight lines. This should not cause surprise by its arbitrariness. Other arguments advanced as to angle of contact and looseness of packing would, of course, strengthen rather than weaken the evidence already afforded that pressure deficiencies at high moistures could exceed  $4.5 T/r$ .

#### SUMMARY.

A recapitulation of the main features of the moisture distribution in an ideal soil is given in order to emphasise a point previously neglected, namely, that the changes are not in the main strictly reversible, but fall into two series corresponding to the two directions of moisture change. The cellular nature of the soil pore space imposes a quantum character on the moisture changes over a great part of the higher moisture range. The individual cell does not fill or empty by smooth reversible changes but shows two unstable stages at which filling or emptying is completed at a bound. For falling moisture the suction level is that at which a meniscus can invade a cell through one of its narrow entries, which gives a value in the neighbourhood of  $12 T/r$  for close packing. For rising moisture the suction level is that for which water returns to the cell by the collapse of a bubble in it, giving a lower value in the neighbourhood of  $6.9 T/r$ . A still lower value of  $4.5 T/r$  can be reached for a particular type of distribution confined to the lower moisture range.

All the above suction values are closely verified by measurements made with bronze balls and paraffin oil. A detailed exploration of the case for glistening dew and water has been made, which verifies the theory while illustrating more nearly the behaviour of an irregular soil. The two values which rule the two halves of the "hysteresis loop" for this case of natural or common packing are  $6.0 T/r$  for falling moisture and  $4.0 T/r$  for rising.

The new considerations resolve the differences of opinion raised by

R. A. Fisher. It appears that the author has throughout supported a case which belongs correctly to the falling moisture series. Fisher considered a very limited series for the rising moisture case and a hypothetical reversible series, and has been in error in so far as his treatment was regarded as exclusive.

## REFERENCES.

- (1) HAINES, W. B. *J. Agric. Sci.* (1925), **15**, 529.
- (2) ——— *J. Agric. Sci.* (1927), **17**, 264.
- (3) FISHER, R. A. *J. Agric. Sci.* (1926), **16**, 492.
- (4) ——— *J. Agric. Sci.* (1928), **18**, 406.
- (5) VERSLUYS, J. *Int. Mitt. für Bodenkunde* (1917), **7**, 117; also in Mitscherlich's *Bodenkunde*, 131.
- (6) GREEN, H. and AMPT, G. A. *J. Agric. Sci.* (1912), **5**, 1.
- (7) HACKETT, F. E. *Trans. Faraday Soc.* (1922), **17**, Pt 2, 260.
- (8) HACKETT, F. E. and STRETTAN, J. S. *J. Agric. Sci.* (1928), **18**, 671.
- (9) DE WITT, C. C. and BROWN, G. G. Colloid Symposium Monograph (1927), **5**, 313.

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# BIOCHEMISTRY OF WATERLOGGED SOILS. PART III<sup>1</sup>.

## DECOMPOSITION OF CARBOHYDRATES WITH SPECIAL REFERENCE TO FORMATION OF ORGANIC ACIDS.

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(With Eight Text-figures.)

IN the previous communications(25, 26) the more prominent effects of waterlogging in absence of foreign materials were described. The present paper deals with a study of the changes that attend addition of nitrates and carbohydrates.

### EFFECT OF ADDITION OF NITRATES.

Warington's experiment(29) on percolation of nitrates through waterlogged soil was repeated with a view to determining whether any denitrification occurred under such conditions. Nitrates, after collection, were estimated by the Devarda alloy method(21). It was observed that in absence of plant residues nitrates could be completely recovered by repeated percolation, though the high concentration made the soil sticky and difficult to work with.

Table I.

Nitrates as p.p.m. of nitrogen.							
Exp. No.	Soil	Days ...	0	1	3	5	7
I	Rothamsted	...	18.4	17.4	20.1	17.2	20.0
	Indian ...	...	18.4	16.9	15.4	13.6	18.8
II	Rothamsted	...	36.7	35.2	32.5	31.4	35.1
	Indian ...	...	36.7	32.7	26.5	29.1	30.4
III	Rothamsted	...	55.2	53.9	50.8	52.7	56.9
	Indian ...	...	55.2	49.5	47.2	52.6	47.7
IV	Rothamsted	...	73.5	72.1	74.8	72.3	71.9
	Indian ...	...	73.5	68.4	72.4	66.9	65.5
V	Rothamsted	...	92.1	83.8	78.7	81.2	76.1
	Indian ...	...	92.1	79.5	79.8	81.5	79.0

Average diminution ( $\bar{x}$ ) ÷ standard error =  $t^{(6)}$ ;  $t$  for 0.1 day = 3.5, which is significant.

In order to determine whether concentration of the nitrate had any effect on its subsequent transformation in the waterlogged soil, potassium nitrate was added in aqueous solution to 100 gm. lots of the same soils as those previously studied, to correspond to different concentrations

<sup>1</sup> Part of thesis accepted by the University of London for the degree of Doctor of Science.

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and the mixtures were incubated, the Rothamsted soil at 20° C. and the Indian soil at 35° C., after being waterlogged. Samples were analysed at two-day intervals (Table I).

Though nitrates suffered a slight loss at the end of the 1st day, yet a simultaneous determination of total nitrogen indicated no change, thereby proving that no denitrification had occurred.

### EFFECT OF ADDITION OF FERMENTABLE ORGANIC MATTER.

A study of the transformation of organic matter is of fundamental importance and will throw light on the mode of decomposition of (a) straw, leaves, etc. which occur in the soil, (b) green manures and different other forms of organic fertilisers that are generally allowed to rot in the puddled soil prior to transplanting of paddy, and (c) various mineral transformations resulting in increase of plant food that occurs under such conditions. But it is exceedingly difficult to carry out because of (a) the complex composition of soil, (b) enormous number and variety of soil microflora, each member of which carries out its own characteristic function, and (c) want of adequate technique to deal either with the mixed flora or the different products of their metabolism as obtained under waterlogged conditions.

Among the different methods for studying biological activity in the soil the one introduced by Remy<sup>(21)</sup> and extended by Lohnis<sup>(14)</sup> is defective because the reactions are allowed to proceed in artificial media which do not represent natural soil conditions. The technique of Withers and Fraps<sup>(30)</sup> also introduces abnormal conditions because of the use of sterilised soil. The Lipman-Brown<sup>(13)</sup> and Russell-Hutchinson<sup>(23)</sup> methods are useful in the study of the release of available plant food, the former from added materials and the latter from unavailable forms present in the soil. In the present study an attempt was made to combine the Russell and Lipman methods with some modifications. Where adequate chemical techniques were lacking new ones were introduced. To avoid complexity due to indeterminate compositions of the added materials the following series of trials were carried out mainly with carbohydrates of well-defined composition.

### EFFECT OF ADDED GLUCOSE ON NITRATES.

Glucose was added in solution to correspond to 600, 1200, 1800, 2400 and 3000 parts respectively as carbon per million parts of the soils which were waterlogged as usual<sup>(25)</sup>. Nitrates were determined every 2 days (Table II).

It was observed that even at the end of 24 hours the soils developed a characteristic odour, and were frothy with carbon dioxide, and acid to phenolphthalein even after boiling. At later stages the changes were more pronounced.

Table II.

Nitrates in the Rothamsted and the Indian soils, at the beginning, were 16.1 and 43.8 p.p.m. respectively.

Nitrates as p.p.m. of nitrogen.

Glucose as p.p.m. of carbon	Days ...	Rothamsted soil				Indian soil			
		1	3	5	7	1	3	5	7
None		13.4	12.1	10.8	11.7	39.6	41.1	38.6	40.7
600		6.6	2.1	3.5	1.8	16.4	4.7	3.4	1.8
1200		3.0	...	...	...	6.8	2.1	1.6	...
1800		...	...	...	...	3.2	...	...	...

... nil; 2400 and 3000 p.p.m. of carbon—nil throughout.

In order to observe whether addition of glucose caused corresponding losses in total nitrogen a similar series of determinations were made with 30 gm. lots of soils. The results, however, showed that the changes were at no time significant. It should, therefore, be inferred that the rapid disappearance of nitrates was not due to denitrification but to transformation to other forms of nitrogen.

#### DISSOLVED OXYGEN.

In order to determine whether addition of sugar introduced any change in the oxygen-contents, trials were carried out under the same conditions as in the previous experiments. Since, owing to the constant interchange of oxygen between the soil-sediment and the surface water (25), an increase or decrease in the one would be correspondingly reflected in the other, it was considered sufficient to determine only the amounts of oxygen present in surface water. Rideal and Stewart's modification (22)

Table III.

Oxygen at the beginning in both soils = 7.3 p.p.m.

Dissolved oxygen as p.p.m.

Glucose as p.p.m. of carbon	Days ...	Rothamsted soil				Indian soil			
		1	3	5	7	1	3	5	7
None		7.9	8.7	7.0	8.4	5.8	6.1	5.2	5.6
600		1.6	5.3	5.8	8.0	1.1	2.4	5.3	6.3
1200		1.3	2.8	3.7	4.9	1.4	1.2	0.2	2.4
1800		1.0	1.1	2.1	2.6	0.7	1.0	1.3	1.7
2400		0.5	0.4	1.3	1.7	0.3	0.4	0.8	1.0
3000		0.2	nil	0.4	1.0	nil	0.2	0.6	0.6

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of Winkler's method was adopted with correction for dissolved reducing matter. The results (Table III) showed that the oxygen-contents first fell considerably, but soon rose owing to diffusion of oxygen from air. The recovery was rapid when the sugar added was small, but slow when large.

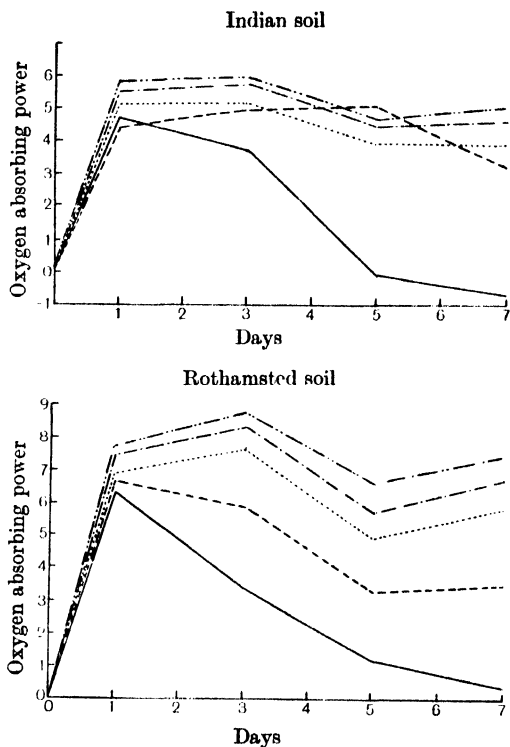


Fig. 1. Oxygen absorbing power.

———— Soil + 600 p.p.m. of carbon.      - - - - - Soil + 1200 p.p.m. of carbon.  
 ..... Soil + 1800                      - · - · - Soil + 2400                      "  
 — · — · — Soil + 3000 p.p.m. of carbon.

The addition of sugar led evidently to some internal reaction which caused rapid depletion of oxygen. At later stages oxygen of the air diffused in and restored normal conditions. Since the soils remained exposed to air, oxygen in accordance with the gas laws would have diffused into them only in proportion to their shortage from the normal. "Oxygen absorbing powers" of the soils thus estimated (Fig. 1) measured also, indirectly, the intensity of biological action that was mostly, if not entirely, responsible for the depletion of oxygen.

*Carbon dioxide* present, dissolved in the surface water, was determined

by absorption in baryta and back-titration against standard acid. The data (Fig. 2), though not absolute measures of the gas produced, were estimates of the concentrations developed at different stages. They are positively correlated to the corresponding figures for oxygen absorbing power and indicate that oxygen was largely utilised for the biological oxidation of the carbohydrate to carbon dioxide.

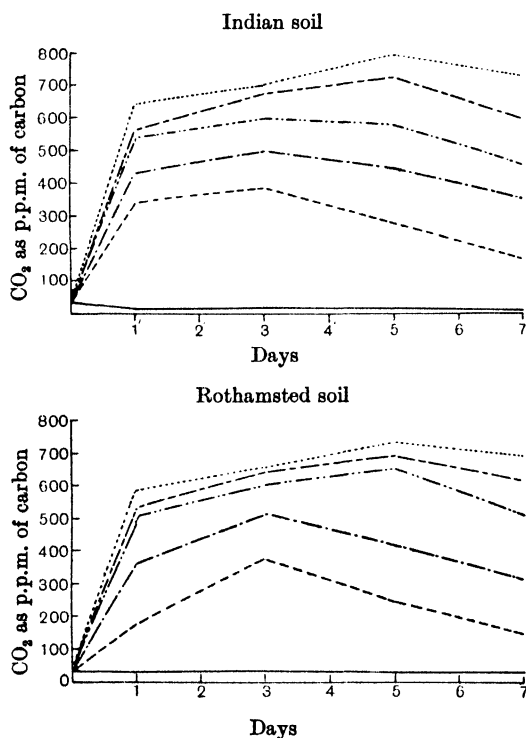


Fig. 2. Dissolved carbon dioxide.

————	Soil alone.		
-----	" + 600 p.p.m. of fermentable carbon.		
- . . . - . . .	" + 1200	"	"
— . . . — . . .	" + 1800	"	"
-----	" + 2400	"	"
.....	" + 3000	"	"

The reaction of aqueous extracts of soils to which sugar had been added, as determined by Gillespie's method (8), indicated a slight initial change (*pH* 7.5 to 6.5) to acidity. It returned to normal on standing.

Tests were carried out with clarified extracts of soils for the presence of organic acids. Lactic acid was detected by its reduction of permanganate and chromic acid with evolution of acetaldehyde, and by

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iodoform, and Fletcher and Hopkins' (7) tests. Acetic and butyric acids were also identified in the liquid by a variety of tests (5).

### BACTERIAL NUMBERS.

Bacterial numbers were determined by plating on Thornton's medium (28). It was observed (Fig. 3) that in addition to the great

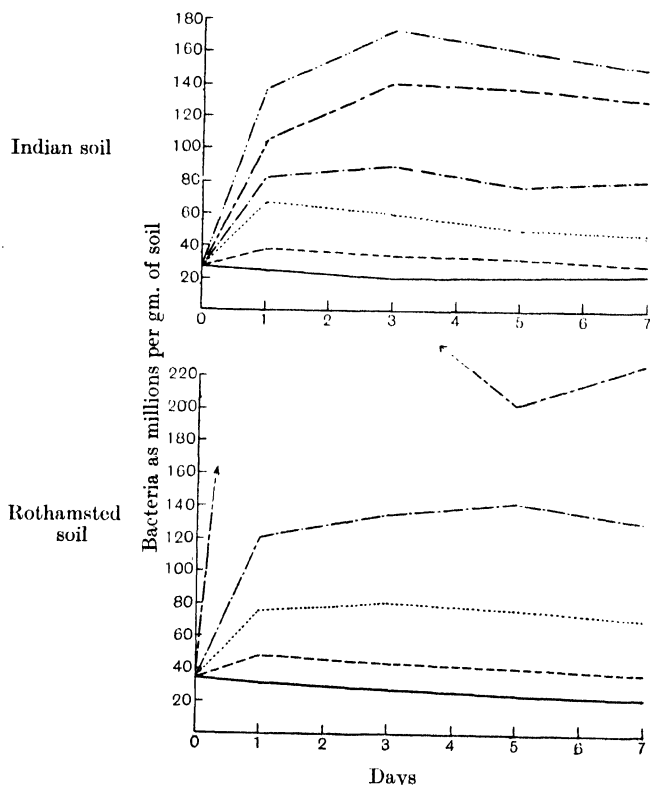


Fig. 3. Bacterial numbers.

Soil alone.                      Soil + 1800 p.p.m. of carbon.  
 " + 600 p.p.m. of carbon.      " + 2400 "  
 " + 1200 "                      " + 3000 "

increase in bacteria, higher concentrations (2400 and 3000 p.p.m.) of the sugar brought out enormous numbers of fungi which rendered counting difficult. The decrease in numbers noticed after the 3rd day might have been due to both shortage of the sugar and to the distinctly visible increase in protozoa.

Correlation between bacterial and oxygen absorption numbers, though positive, was not very close, thereby suggesting that all the bacteria did not depend on oxygen. Many of those responding to the higher concentrations of the sugar might have been either strict or facultative anaerobes. The closer agreement observed between bacterial numbers and CO<sub>2</sub>-production lent further support to this view.

#### ASSIMILATION OF NITRATES BY SOIL ORGANISMS IN PRESENCE OF GLUCOSE.

Soils treated with 600 p.p.m. of the sugar were plated out on Giltay's Agar. After incubation for 7 days at 35° C. the colonies coming out on the plates were examined. The organisms common to all of them, but morphologically different from each other, were inoculated into sterile suspensions of both the soils containing identical amounts of sugar and nearly the same quantities of nitrate by the necessary additions and developed as seed cultures. After they had developed for 24 hours they were transferred into larger quantities of similar soil suspensions. The specimens were incubated for 3 days at 35° C. and then analysed for their nitrate contents (Table IV).

Table IV.

Designation of the organism used	Rothamsted soil		Indian soil		Differences between the amounts used up from two soils
	Present after 3 days	Amount used up	Present after 3 days	Amount used up	
A (R)	28.4	42.9	36.6	36.0	+6.9
B	34.1	37.2	41.8	30.8	+6.4
C	37.3	34.0	45.0	27.6	+6.4
D	67.6	3.7	69.2	3.4	[+0.3]
E	23.3	48.0	32.7	39.9	+8.1
A (I)	40.8	30.5	51.3	21.3	+9.2
B	22.7	48.6	30.1	42.5	+6.1
C	69.4	1.9	68.7	3.9	[-2.0]
D	20.9	50.4	30.3	42.3	+8.1
E	27.2	44.1	35.6	37.0	+7.1

Nitrate originally present in R (Rothamsted soil) = 71.3 p.p.m. and  
I (Indian soil) = 72.6 p.p.m.

Most of the samples had then turned cloudy with bacterial growth. Some of them had developed characteristic odours. No frothing or gas-production was visible. Except in one case the extracts were all acid to phenolphthalein even after boiling.

The results show that (a) nitrate assimilation is common to most soil

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organisms, (b) where the response of an organism to nitrate is pronounced, the effect of the soil substrate is about the same as instanced by the nearly constant differences ( $7.3 \pm 1.1$ ) observed between the amounts of nitrate assimilated in presence of the two soils, and (c) the study of single species of organisms, however prominent, will not be representative: further investigation should, therefore, be carried out with the mixed microflora of the soils.

#### RELATION BETWEEN CONCENTRATION OF NITRATE AND THE RATE OF ITS DECOMPOSITION IN PRESENCE OF GLUCOSE.

100 gm. suspensions of the soils containing 600 p.p.m. of glucose but different proportions of nitrate were incubated, the Rothamsted soil at 20° C. and the Indian soil at 35° C., for 24 hours. They were then analysed for their nitrate-contents and the quantities decomposed by the microflora in each case determined (Table V).

Table V.

Nitrates as p.p.m. of nitrogen.			
Rothamsted soil		Indian soil	
Originally present	Decomposed	Originally present	Decomposed
16.1	9.3	43.8	26.7
34.5	19.4	62.2	33.6
52.8	32.1	80.5	41.1
71.3	49.3	99.0	50.8
89.6	65.5	117.3	51.4
108.2	65.1	—	—

The rate of decomposition of nitrate increases with concentration up to a maximum. It is higher for the Rothamsted soil than for the Indian in spite of the lower temperature of incubation and bears evidence to the greater biological activity in the former.

#### DISTRIBUTION OF CARBON AND NITROGEN IN WATERLOGGED SOILS.

Simultaneous changes in different forms of nitrogen and carbon were studied, using suspensions of soils containing glucose (600 p.p.m.) and nitrate (89.6 p.p.m. in the Rothamsted and 99.0 in the Indian specimens respectively). The soils were waterlogged and incubated under conditions similar to those in the previous series for 48 hours and samples taken for analyses every 12 hours. The determinations were not carried out over a longer period because it was observed that most of the changes took place within 2 days.

Residual sugar at different stages was estimated by a modified Fehling titration method(27): total  $\text{CO}_2$  by absorption in baryta, with boiling to drive off that present in the surface water: nitrite by the Davisson method(4): nitrate by the Devarda alloy method(24): ammonia

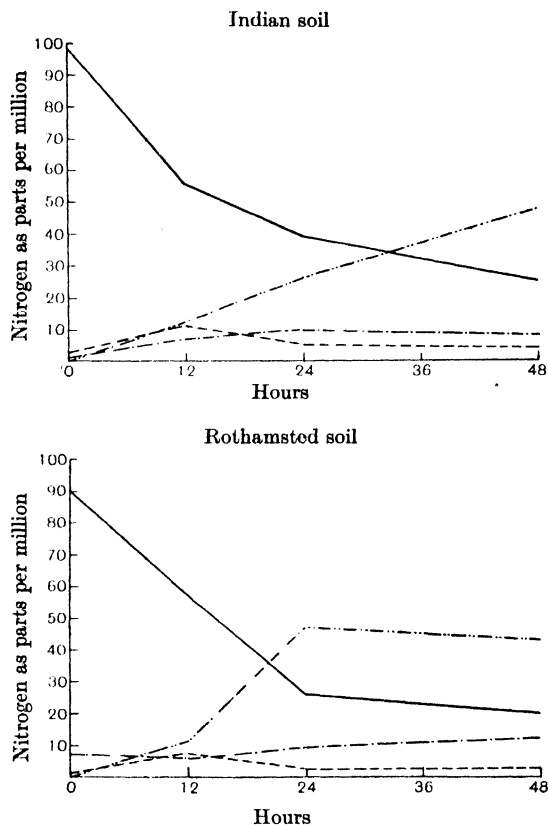


Fig. 4. Distribution of nitrogen.

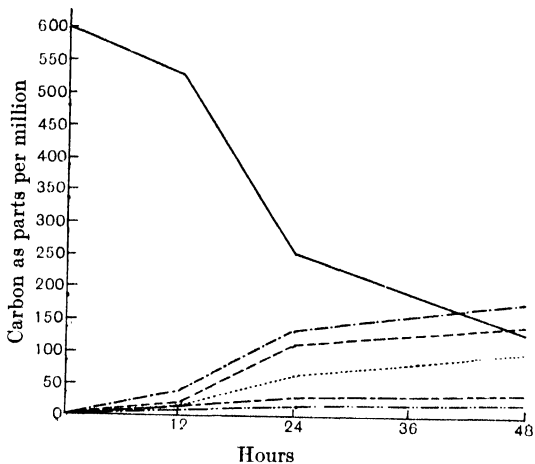
———— Nitrogen as nitrates.      - - - - - Nitrogen as nitrites.  
 - . . . . " as ammonia.      . . . . . " assimilated by microflora.

by the McLean and Robinson method(19): total nitrogen by the Gunning-Hubbard method(1): lactic acid by the Buchner and Meisenheimer method(3) after concentrating the neutralised aqueous extract to a small volume: and acetic and butyric acids by steam-distilling at constant volume(27).

## CHANGES IN DIFFERENT FORMS OF NITROGEN (Fig. 4).

As was expected nitrates disappeared rapidly: nitrites increased at the same time, suggesting that they were formed mainly from them.

Indian soil



Rothamsted soil

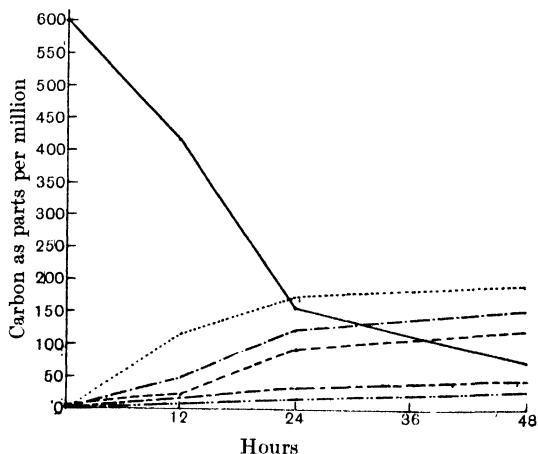


Fig. 5. Distribution of carbon.

— Carbohydrate added.      - - - - - CO<sub>2</sub> produced.  
 - . . . . Lactic acid formed.      - - - - - Acetic acid formed.  
 - . . . . Butyric acid formed.      . . . . . Carbon taken up by micro-organisms.

Formation of nitrite accords with the observations of Nagaoka (20), Kelly (11) and others. The subsequent decrease in nitrites may have been

due to (a) reduction to ammonia, (b) direct assimilation by micro-organisms, and (c) denitrification. Since total nitrogen remained unaltered throughout the period under observation it should be inferred either that there was no loss by denitrification, or that it was too small to be appreciable. There was significant increase in ammonia in both soils which may have been due to (a) the action of the deaminising enzyme present in the soils<sup>(26)</sup>, and (b) reduction of nitrates and nitrites by ammonifiers<sup>(17)</sup>.

Nitrogen taken up by micro-organisms or otherwise converted into more complex forms was taken to be approximately the difference between the sums of nitrate, nitrite and ammonia present in the beginning and at the end. The figures showed that the major part of the added nitrogen was thus converted. Since the diminution in the quantities of the nitrates and nitrites present occurred simultaneously with the increased uptake by the micro-organisms and since no other changes were noticeable, it appeared that most of the two soluble forms were thus converted. Since nitrogen of cells of micro-organisms is essentially protein it seems probable that most of the transformed nitrogen was converted into that form.

#### CHANGES IN DIFFERENT FORMS OF CARBON (Fig. 5).

In both soils the decomposition of the added sugar was extremely rapid. About one-fourth to one-fifth of the total added carbon passed into  $\text{CO}_2$ . Production of lactic acid accounting for between 25 and 30 per cent. of sugar in both soils was an important change, and may have been due largely to lactic acid bacteria and, to a limited extent, to certain putrefactive and saprophytic organisms like *B. coli* and *B. lactis aerogenes* present in soil. Only small quantities of acetic and butyric acids were formed during the period under investigation. Their molecular proportion was approximately as two of acetic to one of butyric. Quantities of the added carbon converted into complex forms by cells of micro-organisms or otherwise were taken as represented by differences between initial and subsequent amounts present in simpler forms. The figures show that such transformations are greater in Rothamsted than Indian soil. No definite relationship could be found between carbon and nitrogen assimilations.

#### GENERAL DISTRIBUTION OF THE DECOMPOSED CARBON.

Dividing carbon changes under four heads—(a) total converted, (b) passing into gas, (c) taken up by micro-organisms, (d) passing into organic acids—it is evident that the last change is not only unique to the

waterlogged soil, but also the most prominent, involving nearly half the added carbon. The production of acids, particularly lactic, though beginning comparatively slowly, soon proceeded at a rapid rate. Even at the end of 48 hours there was no indication of the reaction weakening.

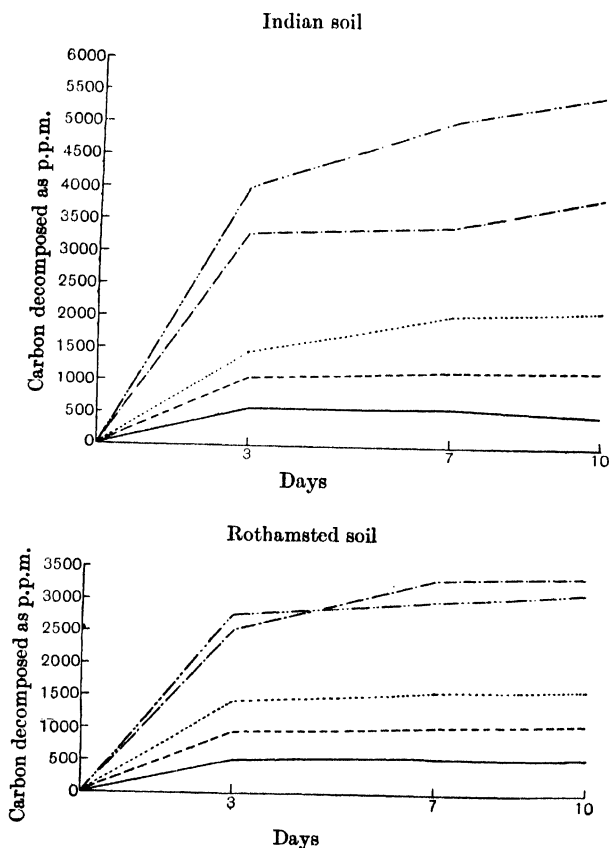


Fig. 6. Effect of concentration on decomposition of fermentable carbon.

_____ 600 p.p.m. added carbon.	_____ 6000 p.p.m. added carbon.
----- 1200                   "	----- 12,000                   "
..... 2400 p.p.m. added carbon.	

Since, however, it is known that many of the common bacteria and fungi can not only assimilate organic acids and their salts but also decompose them into simpler compounds(16), the presence of large quantities of organic acids would naturally mean that further biological activity would follow later on. Acid-production would thus be only an intermediate stage in a long series of reactions.

DECOMPOSITION OF ADDED CARBOHYDRATE AT DIFFERENT  
CONCENTRATIONS.

To specimens of Rothamsted and Indian soils glucose was added from aqueous solution to correspond to 600, 1200, 2400, 6000, and 12,000 p.p.m. of carbon, and after waterlogging incubated for 10 days at 20° C.

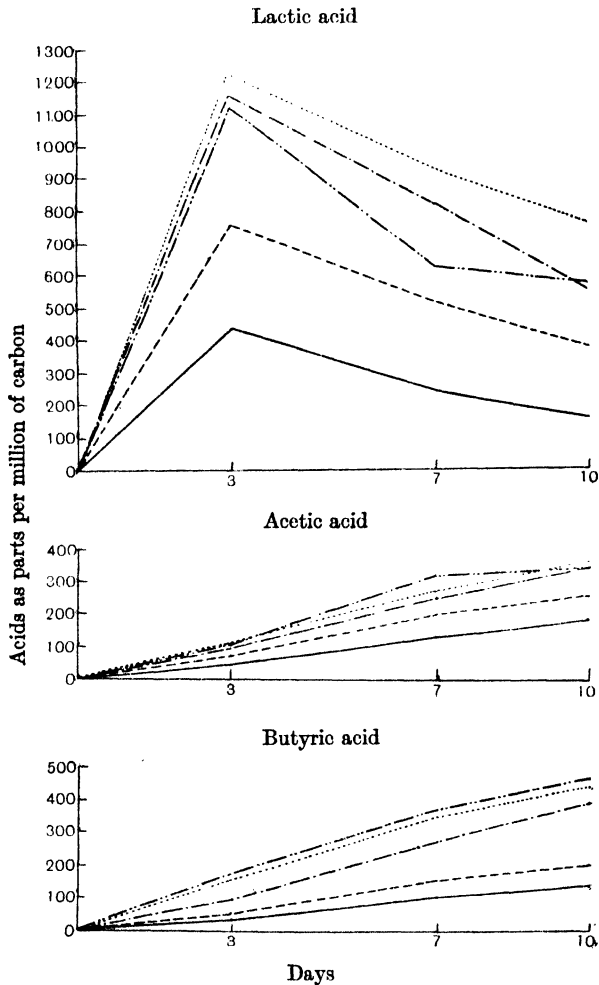


Fig. 7. Formation of lactic, acetic and butyric acids—Rothamsted soil.

—————	2400 p.p.m. fermentable carbon.
- - - - -	4800 " "
- . . . .	7200 " "
.....	9600 " "
- . . . .	12,000 " "

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and 35° C. respectively. Samples were analysed every 3 days for their sugar-contents(27).

The results (Fig. 6) showed that decomposition of sugar of all concentrations proceeded vigorously up to the end of the 3rd day, after which there was general slackening. This was probably due to shortage of sugar at lower concentrations and accumulation of acids and other products of biological metabolism at higher ones. Total quantities decomposed were, proportionately, much less at higher concentrations than at lower ones.

### PRODUCTION OF ORGANIC ACIDS AT DIFFERENT CONCENTRATIONS OF ADDED CARBOHYDRATE.

Soils were treated in the same manner as in the previous trial. Lactic acid was estimated by Long's method(15) and acetic and butyric acids by steam-distillation at constant volume(27). Similar types of results were obtained for both soils. Those for Rothamsted soil are plotted in Fig. 7.

At all concentrations of sugar lactic acid was the first product of metabolism and accounted for 30-40 per cent. of the sugar decomposed. After the 3rd day, however, lactic acid tended to diminish, being, evidently, converted into other forms.

Acetic and butyric acids were formed slowly but continuously throughout the period under observation. There was no relation between their quantities and the corresponding amounts of sugar decomposed. Molecular proportions of the two acids were approximately as two of acetic to one of butyric. There was high positive correlation between decrease in lactic and corresponding increase in the other two acids, suggesting that decomposition of the former led, at least partly, to formation of the latter.

Table VI.

Amounts of acids formed as p.p.m. of carbon.

Depth of water	Time in days	Lactic	Acetic	Butyric
Soil just covered	3	912	192	72
	7	516	384	216
	10	264	456	264
2"	3	1164	84	144
	7	432	312	324
	10	336	408	432
4"	3	1272	60	168
	7	684	288	384
	10	432	432	456

RELATION BETWEEN DEPTH OF WATER AND PRODUCTION  
OF ORGANIC ACIDS.

Specimens of Rothamsted soil containing 6000 p.p.m. of glucose were suspended under different depths of water and the acids formed at the end of 3, 7 and 10 days estimated (Table VI).

Greater depths led to larger production of lactic and butyric acids, suggesting that their formation did not depend on free supply of air. Diminished production of acetic acid under similar conditions suggested the reverse.

EFFECT OF ADDITION OF CALCIUM CARBONATE ON  
PRODUCTION OF ACIDS.

To 30 gm. portions of the soils containing 6000 p.p.m. of glucose, 2 gm. of calcium carbonate were added before waterlogging. The acids formed were estimated at the end of 3, 7 and 10 days (Table VII).

Table VII.

Treatment	Time in days	Amounts of acids as p.p.m. of carbon.					
		Rothamsted soil			Indian soil		
		Lactic	Acetic	Butyric	Lactic	Acetic	Butyric
Without $\text{CaCO}_3$	3	1164	100	95	1224	118	139
	7	816	252	276	494	384	516
	10	552	348	396	256	432	624
With $\text{CaCO}_3$	3	1536	118	121	1788	152	166
	7	745	334	451	991	387	494
	10	374	392	506	716	451	577

Addition of calcium carbonate led to distinct increase in lactic acid: subsequent decomposition of the acid was also rapid, particularly in Rothamsted soil.

The effect of carbonate on the production of the other acids was not pronounced.

PRODUCTION OF ORGANIC ACIDS DURING AN EXTENDED  
PERIOD OF OBSERVATION.

Specimens of the Rothamsted soil containing 6000 p.p.m. of glucose were analysed for their acid-contents at intervals during a period of 30 days.

It was observed (Fig. 8) that lactic acid decomposed actively and fairly uniformly. Acetic and butyric acids, after increasing till about the 15th day, decomposed appreciably at later stages. The results support

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the theory, already suggested, that the acids would, on standing, be attacked by micro-organisms and converted into other forms.

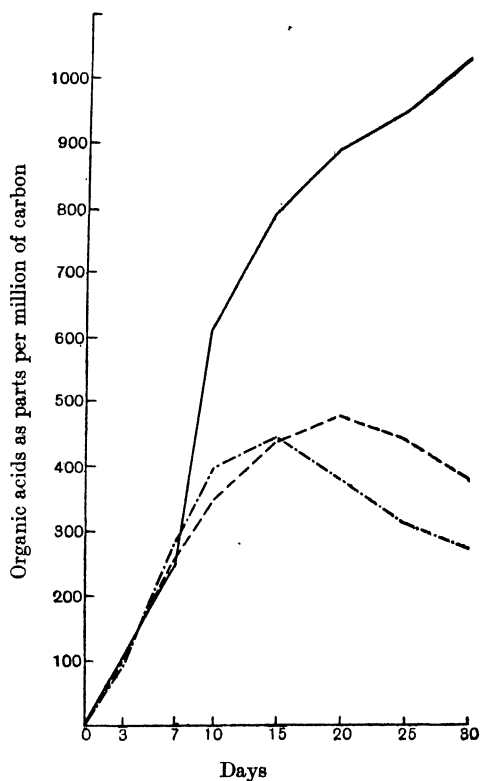


Fig. 8. Changes in organic acids over a long period.

————— Lactic acid decomposed.      - - - - - Acetic acid produced.  
 - . - . - Butyric acid produced.

### PRODUCTION OF LACTIC ACID UNDER AEROBIC AND ANAEROBIC CONDITIONS.

20 gm. lots of Rothamsted soil were waterlogged with 4000 p.p.m. of glucose. In one set the mixture was allowed to stand in shallow, glass-covered trays. In the other, after being made up with air-free water, it was placed in narrow-necked flasks connected with U-tubes containing alkaline pyrogallol. Both sets were maintained at the ordinary temperature (20–25° C.). Lactic acid present on successive days was estimated (Table VIII).

Table VIII.

Lactic acid formed as p.p.m. of carbon.

Time in days	Aerobic		Anaerobic	
	Control soil and water alone	With the sugar	Control soil and water alone	With the sugar
1	27	378	45	567
2	54	907	63	855
3	45	1134	54	1071

Production of lactic acid occurred under both aerobic and anaerobic conditions and to about the same extent.

## FORMATION OF LACTIC ACID IN ABSENCE OF LIVING ORGANISMS.

20 gm. lots of Rothamsted soil were weighed out into dishes with glass covers and allowed to remain soaked in toluene at the ordinary temperature for 48 hours, after which 50 c.c. of distilled water and 20 c.c. portions of 1 per cent. solutions of glucose, sucrose and lactose respectively were added to them. The mixtures were allowed to stand for 3 days. Lactic acid in this and the subsequent series was estimated by the author's modification (27) of the chromic acid method (Table IX).

Table IX.

Lactic acid formed as p.p.m. of carbon.

Time in days	Control soil alone	Glucose	Sucrose	Lactose
1	27	108	189	72
2	54	207	243	189
3	54	234	297	216

It is probable that part of the lactic acid formed during waterlogging was enzymic in origin. The major part, however, was evidently formed by the activity of living organisms.

## MECHANISM OF PRODUCTION OF LACTIC ACID.

Since the acid was formed under both aerobic and anaerobic conditions, its production did not involve the utilisation of atmospheric oxygen. Nor is it probable that any biological reduction of the sugar was involved since the acid is a more highly oxygenated product than the sugar. The chemical mechanism was probably either hydrolysis or direct splitting of the carbohydrate. Though Hoppe-Seyler (10) and later Kiliani (12) showed that lactic acid can be formed by the hydrolysis of certain carbohydrates, yet the conditions of their experiments bear no analogy to the production of the acid in the waterlogged soil. Since it

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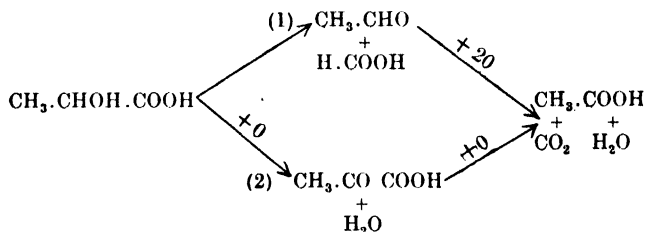
was also observed on trial that the acid was not formed by acid-hydrolysis of the sugars even at  $100^{\circ}\text{C}$ . it may be inferred that hydrolysis was not the mechanism of its production. Biological production of lactic acid was therefore probably one of fission of the carbohydrate molecule which did not bear any relation to the reactions brought about by common chemical reagents. Formation of the acid from glucose would then be represented in outline by  $\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2\text{C}_3\text{H}_6\text{O}_3$ . The primary change involved in its production would therefore appear to be intramolecular rearrangement of the sugar.

### PRODUCTION OF ACETIC AND BUTYRIC ACIDS FROM LACTIC ACID.

In order to test whether any of the fatty acids were formed directly from lactic acid, quantities of the latter corresponding to 1200 p.p.m. of carbon were added to the Rothamsted soil and waterlogged under aerobic and anaerobic conditions. The former was effected by gentle bubbling of air through the suspension and the latter by using air-free water and connecting the flasks to U-tubes containing alkaline pyrogallol. The fatty acids were identified and estimated at intervals of 24 hours (Table X).

The extent of decomposition of lactic acid was about the same under aerobic and anaerobic conditions. Under anaerobic conditions the greater part of it passed into fatty acids, while only less than half did so under the aerobic.

The quantities of acetic acid produced under aerobic conditions were uniformly greater than those produced anaerobically. In the former it was formed almost to the entire exclusion of butyric acid and appeared to have been mainly due to oxidation of lactic acid which might have been through the stages of (1) acetaldehyde or (2) pyruvic acid. Both types of reactions might also have taken place simultaneously.



Qualitative analyses made from time to time showed only the presence of pyruvic acid, as indicated by the nitroprusside,  $\beta$ -naphthol and phenylhydrazine reactions. Positive reactions for the aldehyde

could at no time be obtained. As shown by Maze and Ruot(18) it is probable that certain fungi brought about the oxidation of lactic into pyruvic acid.

Table X.

Time in days	Acids as p.p.m. of carbon.					
	Aerobic			Anaerobic		
	Lactic	Acetic	Butyric	Lactic	Acetic	Butyric
1	984	96	24	996	48	132
2	816	264	48	744	168	276
3	552	216	36	624	192	336

Attempts at reproducing the chemical oxidation of lactic acid at concentrations similar to those obtained in the waterlogged soil to pyruvic acid by the Aristoff method(2) resulted only in the formation of acetaldehyde as the intermediate product. The biological formation of pyruvic acid which proceeds under nearly neutral conditions (pH 6.5) has evidently a different mechanism.

Production of the fatty acids seems to proceed along different lines under anaerobic conditions. Neither pyruvic acid nor aldehyde could be detected at any time.

The molecular proportion under fully aerobic conditions was as 12 of acetic to 1 of butyric and, under anaerobic, approximately as 1 : 1. Since in the waterlogged soil it was 2 : 1 it should be inferred that the conditions therein were intermediate between the above two.

#### ORGANISMS RESPONSIBLE FOR THE DIRECT ASSIMILATION OF LACTIC ACID.

To isolate the organisms directly assimilating lactic acid the soils were plated out on a medium containing lactic acid (0.2 per cent.), soil extract (sp. gr. 1.002), agar (2.0 per cent.) and a few drops of brom-cresol-purple indicator. After incubation for 10 days at the ordinary temperature the organisms turning the medium purple (*i.e.* destroying the acid and thereby rendering the medium neutral) were examined.

The plates from the Rothamsted soil contained a large number of colonies of a micrococcus which produced the purple within a week. There were also rapidly growing colonies of a *Penicillium* which did not produce the colour till the 10th day. Plates from the Indian soil also contained the *Penicillium*, but the coccoid forms were not to be found. On inoculating the two organisms into lactic acid (45 milligrams) diluted with soil extract (100 c.c., sp. gr. 1.002) it was observed that the acid was rapidly assimilated (Table XI).

Table XI.

Lactic acid assimilated in mg.				
Organism			3 days	7 days
<i>Micrococcus</i>	...	...	9.7	14.1
<i>Penicillium</i>	...	...	12.0	28.7

No acetic or butyric acid was formed at the same time. Qualitative examination showed that the mucilaginous development of the coccus was a carbohydrate resembling glycogen in iodine reaction.

#### PRODUCTION OF ORGANIC ACIDS FROM DIFFERENT CARBOHYDRATES.

Specimens of the Rothamsted soil were treated with (1) glycerol, (2) xylose, (3) arabinose, (4) laevulose, (5) mannitol, (6) sucrose, (7) maltose, (8) lactose, (9) starch, (10) maltodextrin and (11) cellulose (cotton-wool), each in quantities corresponding to 6000 p.p.m. of carbon, waterlogged at the laboratory temperature (20–25° C.). The acids formed at different stages were estimated (Table XII).

Table XII.

Organic acids as p.p.m. of carbon.

No.	Time in days	Lactic	Acetic	Butyric
1	3	67	35	82
	7	112	58	61
2	3	314	46	76
	7	88	118	62
3	3	1126	200	204
	7	272	498	292
4	3	978	272	331
	7	421	326	484
5	3	1187	18	278
	7	321	86	543
	10	119	401	446
6	3	679	126	320
	7	214	333	513
7	3	1688	540	291
	7	796	762	161
8	3	892	306	112
	7	264	416	268
9	3	382	102	101
	7	204	128	62
10	3	436	115	251
	7	196	85	234

Minute quantities of the acids were also formed from cellulose. Formation of the acids was generally accompanied by production of carbon dioxide. The quantities of acetic and butyric acids did not bear

any relation to each other or to decomposed lactic acid. Though the same acids were formed in every case their modes of formation appeared to vary.

#### SUMMARY.

(1) In absence of decomposing organic matter addition of nitrate led to no loss of nitrogen.

(2) On addition of small quantities of fermentable matter such as glucose there was (a) rapid depletion of nitrates and oxygen, but no denitrification, and (b) increase in acidity, carbon dioxide and bacteria. The greater part of the soluble nitrogen was assimilated by micro-organisms or otherwise converted and the greater part of the added carbohydrate was transformed into lactic, acetic and butyric acids.

(3) The organic acids were formed from a variety of carbohydrates. Lactic acid was the first to be observed and appeared to be formed mainly by direct splitting of the sugar. It decomposed readily, forming acetic and butyric acids. Some acetic acid was formed by direct oxidation of lactic acid, with pyruvic acid as the intermediate product. All the acids were, on standing, converted into other forms by micro-organisms.

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#### REFERENCES.

- (1) J. Assoc. Off. Agric. Chem. *Methods of Analysis* (1925), 27.
- (2) ARISTOFF, V. *J. Russ. Phys. Chem. Soc.* (1884), **2**, 249.
- (3) BUCHNER, E. and MEISENHEIMER. *J. Ber.* (1908), **41**, 1410; (1913), **43**, 1773.
- (4) DAVISSON, B. S. *J. Amer. Chem. Soc.* (1916), **38**, 1683.
- (5) DYER, D. C. *J. Biol. Chem.* (1917), **28**, 445.
- (6) FISHER, R. A. *Statistical Methods for Research Workers*. Oliver and Boyd, 1925.
- (7) FLETCHER and HOPKINS, F. G. *J. Physiol.* (1907), **35**, 247.
- (8) GILLESPIE, L. J. *Soil Sci.* (1920), **9**, 115.
- (9) HARRISON, W. H. and IYER, P. A. S. *Mem. Ind. Agric. Dept. Chem. Ser.* (1913-14), **3**, 65.
- (10) HOPPE-SEYLER, F. *Ber.* (1871), **4**, 346.
- (11) KELLY, W. P. *Hawaii Agric. Expt. Sta. Bull.* (1911), **24**.
- (12) KILIANI, H. *Ber.* (1882), **15**, 699.
- (13) LIPMAN, J. G. and BROWN, P. E. *Cent. f. Bakt.* (1910), Abt. **2**, **26**, 590.
- (14) LOHNIS. *Mitt. Landw. Inst. Univ. Leipzig.* (1905), 1-103.
- (15) LONG, C. H. N. *Proc. Roy. Soc. Lond.* (1924), B, **96**, 444.

## 648     *Biochemistry of Waterlogged Soils. Part III*

- (16) MAASSEN. *Arb. a. d. Kais. Gesundheitsamt* (1896), **12**, 340.
- (17) MARCHAL, E. *Agric. Sci.* (1894), 574.
- (18) MAZE, P. and RUOT, M. *Compt. rend. Soc. Biol.* (1916), **79**, 706.
- (19) MCLEAN, W. and ROBINSON, G. W. *J. Agric. Sci.* (1924), **14**, 548.
- (20) NAGAOKA, M. *Bull. Tokyo Coll. of Agric.* (1904-5), **6**, 285.
- (21) REMY, TH. *Centr. Bakt.* (1902), Abt. 2, **8**, 657.
- (22) RIDEAL, S. and STEWART, C. G. *Analyst* (1901), **26**, 141.
- (23) RUSSELL, E. J. and HUTCHINSON, H. B. *J. Agric. Sci.* (1908-10), **3**, 111;  
(1912-13), **5**, 152. (*Rothamsted Memoirs*, **9**.)
- (24) RUSSELL, E. J. and PAGE, H. J. *Chemists' Year Book* (1926), 806.
- (25) SUBRAHMANYAN, V. *J. Agric. Sci.* (1927), **17**, 429. (*Rothamsted Memoirs*, **13**.)
- (26) — *J. Agric. Sci.* (1927), **17**, 449.
- (27) — *J. Agric. Sci.* (1929), **19**, 649.
- (28) THORNTON, H. G. *Ann. Appl. Biol.* (1922), **9**, 241. (*Rothamsted Memoirs*, **11**.)
- (29) WARINGTON, R. *J. Roy. Agric. Soc. Engl.* (1881), Ser. II, **17**, 333. (*Rothamsted Memoirs*, **5**.)
- (30) WITHERS, W. A. and FRAPS, G. S. *J. Amer. Chem. Soc.* (1901), **23**, 318; (1902), **24**, 528.

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## DETERMINATION OF SOLUBLE CARBOHYDRATES, LACTIC ACID AND VOLATILE FATTY ACIDS IN SOILS AND BIOLOGICAL MEDIA<sup>1</sup>.

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(With One Text-figure.)

IN the course of a study of the decomposition of carbohydrates in water-logged soils(5) special techniques for estimating different products were adopted owing to the ones then available being neither accurate nor directly suited to soil conditions. Since these methods are also applicable to the study of a variety of other problems they have been described in the present paper.

### SOLUBLE CARBOHYDRATES.

The soil was treated with about five times its weight of distilled water saturated with thymol and one-fifth of its weight of alumina cream(6). The mixture was shaken vigorously for 5 minutes in a stoppered bottle and filtered on the Buchner with liberal washing. The filtrate was concentrated to 20–30 c.c. by direct boiling if it contained less than 0.5 gm. sugar, and, *in vacuo*, if more. Humic matter was removed by treatment with basic lead acetate(1) and the excess of the latter removed with sodium sulphate.

When the sugar was above 1 per cent. of the soil, the determination was made by direct titration against Soxhlet's Fehling solution(1) using methylene blue as internal indicator(3). Corrections were made after trials, for (a) sugar retained by soil and basic lead acetate and (b) Fehling reducing matter of soil. Experiments with representative English and Indian soils showed that 97–99 per cent. of added dextrose was recovered and that the reducing matter corresponded to 0.2–0.6 c.c. Fehling solution for every 50 gm. of soil. The errors were constant when identical quantities of soil and clarifying agent were used.

For smaller amounts of sugar, the extract was concentrated to 30–40 c.c., mixed with 50 c.c. of double-strength Fehling solution, and made up to 100 c.c. in a standard flask. Aliquot parts of the mixture were titrated against standard sugar solution. The total amount of sugar originally present in soil was calculated as in back-titration methods,

<sup>1</sup> Part of thesis accepted by the University of London for the degree of Doctor of Science.

after applying the corrections mentioned already. Table I compares data thus obtained for solutions of pure dextrose with those by direct titration.

Table I.

Direct titration			Back titration	
Vol. of dextrose solution (approx. 1 %) taken	Vol. required for 10 c.c. standard Fehling's	Weight of dextrose as mg. per 100 c.c.	No. of c.c. standard sugar required for the titration of 10 c.c. Fehling's sugar mixture	Weight of dextrose as mg. per 100 c.c.
50 c.c.	10.2 c.c.	490.2	—	—
40	12.8	390.6	2.1	389.3
30	16.8	292.6*	4.1	293.2
20	25.6	194.8*	6.2	192.3
15	34.7	145.1*	7.2	144.2

\* From Lane and Eynon's tables. The standard error of difference between the two methods =  $\pm 0.43$  per cent.

Trials carried out by adding known quantities of dextrose to a Rothamsted soil and determining them by back titration, showed that the method was quite accurate (Table II).

Table II.

[Volumes were made up to 100 c.c. in each case. Reducing matter from 50 gm. of soil = 0.6 c.c. of standard sugar solution.]

Standard error of recovery =  $\pm 0.76$  per cent.

Vol. of 1 % dextrose added to 50 gm. soil	Vol. of the Fehling's sugar mixture taken for final titration	Vol. of the standard 0.5 % dextrose solution required	Recovery of added sugar
30 c.c.	10 c.c.	4.2 c.c.	97.1 %
10	20	16.3	96.6
5	25	22.9	96.4
3	50	47.7	98.1

### *Disaccharides.*

Approximately 1 per cent. solutions (100 c.c.) of disaccharides were added to a Rothamsted soil (50 gm.) and determined by the direct-titration method (Table III). Sucrose was inverted by acid hydrolysis prior to titration.

Table III.

Sugar	Control. Vol. of the standard sugar solution required for 100 c.c. Fehling's	Vol. of extract required to reduce 10 c.c. Fehling's	Vol. of sugar solution equivalent to reducing matter from 50 gm. soil	Percentage recovery of added sugar
Maltose	13.4 c.c.	13.9 c.c.	0.9 c.c.	96.3
Lactose	12.8	13.0	0.8	98.0
Sucrose	9.7	9.9	0.5	97.7

Similar trials were carried out by the back-titration method using smaller quantities (5 c.c. to 50 gm. of soil) of the same sugars. The accuracy obtained was  $97 \pm 0.7$  per cent.

#### MEASURING MICROBIAL ACTIVITY.

To test the applicability of the back-titration technique, specimens of soils from I, Barn Field, Plot O, Rothamsted; II, Central Farm, Coimbatore; III, Karjat, Bombay, and IV, Punjab, were each treated with 1 per cent. of their weights of dextrose and the decomposition of the sugar at 20° C. and 15 per cent. moisture studied at intervals. The data obtained indicate the microbiological activities in the soils at different stages.

Table IV.

Soil no.	Sugar present as percentages			
	8 hours	24 hours	48 hours	72 hours
I	84	49.6	11.7	1.4
II	96.4	76.7	49.6	20.4
III	98.5	86.9	65.0	56.5
IV	92.7	62.3	44.2	10.1

#### LACTIC ACID.

This was determined by a modification of Paessler's method (1), since preliminary trials with minute quantities of lactic acid, such as were formed in the water-logged soil, showed (Table V) that complete oxidation of lactic acid to acetic acid did not take place because of the escape of appreciable quantities of the intermediate product, acetaldehyde, into air by bumping during refluxing.

Table V.

Lactic acid taken = 0.03636 gm. 5 c.c. of 1.005 N/10 dichromate + 2 c.c. 1 : 1 sulphuric acid was used for oxidation. Volume made up to 250 c.c. after refluxing.

Water added	Vol. of 1.036 N/50 $\text{Na}_2\text{S}_2\text{O}_3$ solution required for 25 c.c. of final mixture	Lactic acid found
None	18.70 c.c.	0.0247 gm.
50 c.c.	16.95	0.0329
100	17.25	0.0315
150	17.85	0.0287

Experiments carried out at lower temperatures in small glass-covered dishes showed (Fig. 1) that at 35° C. the oxidation of lactic acid to acetic acid proceeded to completion in about 48 hours without any special attention being required. At a lower temperature the reaction was slow whilst at higher ones there was appreciable loss of aldehyde by volatilisation.

To test the applicability of the method, 20 gm. lots of a soil from Kaliganj, Bengal, were treated with glucose in quantities increasing up to 0.2 gm. and the same amount of lactic acid as used in the previous trial. After vigorous shaking with water and filtration, the extracts were rendered slightly alkaline, concentrated by boiling to about 20 c.c., treated with cupric hydroxide(7) and the filtrate, after treatment with

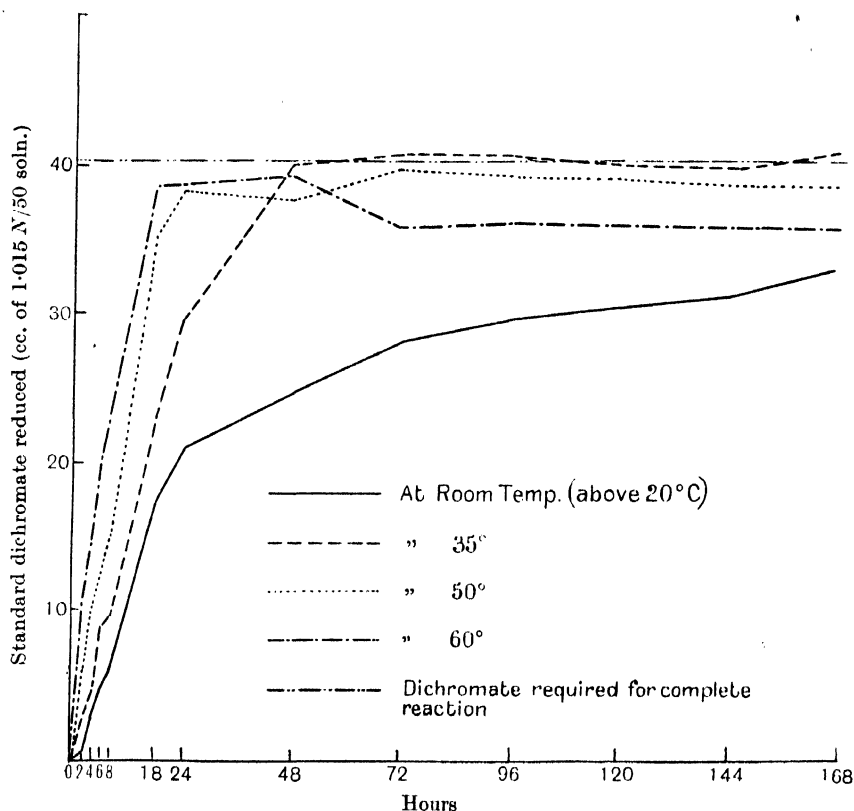


Fig. 1. Quantities of dichromate reacting with lactic acid at different intervals.

dichromate and acid, incubated at 35° C. for two days. Unused dichromate was titrated against thiosulphate iodimetrically.

The figures obtained were identical with those for 35° C. in Fig. 1 showing that (a) lactic acid was completely leached out from the soil with water, (b) cupric hydroxide removed all the reducing sugars, and (c) dichromate oxidation at 35° C. provided an easy and accurate method for determining lactic acid.

## VOLATILE FATTY ACIDS.

These were estimated by a modification of Dyer's method (2) of steam-distillation at constant volume. To test the applicability of the method to such low concentrations as were obtained in the water-logged soils, distillations were carried out with dilute solutions of acetic and butyric acids and the constants for different fractions calculated (Tables VI and VII).

Table VI. *Distilling constants of acetic acid.*

Vol. of acid taken	1st 100 c.c.	2nd 100 c.c.	3rd 100 c.c.	4th 100 c.c.	5th 100 c.c.	6th 100 c.c.
5 c.c.	30.6	30.3	30.1	28.5	26.5	22.2
10	30.9	30.5	30.3	29.9	27.9	26.2
15	31.0	30.5	30.4	30.1	29.6	28.5
25	30.7	30.6	30.5	30.3	30.1	29.7
Means with standard errors	30.8 $\pm$ 0.2	30.5 $\pm$ 0.1	30.3 $\pm$ 0.2	29.7 $\pm$ 0.7	28.5 $\pm$ 1.4	26.7 $\pm$ 2.9

Table VII. *Distilling constants of butyric acid.*

Vol. of acid taken	1st 100 c.c.	2nd 100 c.c.	3rd 100 c.c.	4th 100 c.c.
5 c.c.	70.0	69.1	66.7	—
10	69.7	69.4	68.3	63.2
15	70.1	69.7	68.2	64.3
25	69.8	69.7	69.6	66.7
Means with standard errors	69.9 $\pm$ 0.2	69.5 $\pm$ 0.35	68.2 $\pm$ 0.3	64.7 $\pm$ 1.5

The fractions distilling over became unreliable beyond the third 100 c.c. Average distilling constants at the lower concentrations for the first two 100 c.c. were  $30.6 \pm 0.2$  and  $69.7 \pm 0.3$  for acetic and butyric acids respectively.

Titration figures for mixtures of acetic and butyric acids (Table VIII) agreed with the distilling constants only for the first two 100 c.c. portions.

Table VIII.

		Alkali (1.042 N/50) required					
Vol. of acetic acid taken	Vol. of butyric acid taken	1st 100 c.c.		2nd 100 c.c.		3rd 100 c.c.	
		Calculated c.c.	Found c.c.	Calculated c.c.	Found c.c.	Calculated c.c.	Found c.c.
5 c.c.	25 c.c.	125.0	124.8	42.7	42.2	16.3	14.7
10	15	92.3	92.6	37.7	37.8	18.1	17.3
15	10	82.2	93.0	39.5	38.8	22.0	21.1
25	5	84.5	84.6	49.8	49.3	31.9	31.4
Deviations from those expected		$\pm 0.5\%$		$\pm 1.2\%$		$\pm 5.8\%$	

Table IX shows that the presence of minute quantities of lactic and

sulphuric acids did not appreciably affect the rates of distillation of the fatty acids:

Table IX.

Mixtures containing 10 c.c. each of approximately 1 per cent. solutions of acetic and butyric acids were taken. The expected titration figures for the 1st and 2nd 100 c.c. were 69.8 and 30.9 c.c. respectively of 1.042 *N*/50 alkali.

Alkali (1.042 *N*/50) required.

Vol. of 0.5 % lactic acid added	1st 100 c.c.	2nd 100 c.c.	Vol. of 1 : 8 H <sub>2</sub> SO <sub>4</sub> added	1st 100 c.c.	2nd 100 c.c.
5 c.c.	70.1 c.c.	31.1 c.c.	0.5 c.c.	69.8 c.c.	30.8 c.c.
10	70.0	30.4	1.0	70.2	30.9
15	69.7	30.7	2.0	70.3	31.0
25	70.2	31.0	3.0	70.5	31.3
Deviations from figures expected	±0.4 %	±0.9 %	—	±0.7 %	±0.6 %

*Extraction and determination.*

To 25 gm. lots of six specimens of soils acetic and butyric acids were added. After being treated with about 200 c.c. of distilled water, the suspensions were shaken and filtered with liberal washing. The filtrate after neutralisation was concentrated to about 100 c.c., just acidified with 1 : 8 H<sub>2</sub>SO<sub>4</sub> and steam distilled. After titrating the fractions the actual amounts of acids extracted were calculated thus: Let  $x$  represent the amount of acetic acid and  $y$  that of butyric acid present in the extracts, expressed in terms of the alkali required for neutralisation. Let  $d_1$  and  $d_2$  be the distilling constants for acetic and butyric acids and  $t_1$  and  $t_2$  the titration figures for the first and the second 100 c.c. lots respectively. Then

$$x \frac{d_1}{100} + y \frac{d_2}{100} = t_1 \quad \dots\dots(1),$$

$$x \frac{d_1(100 - d_1)}{100^2} + y \frac{d_2(100 - d_2)}{100^2} = t_2 \quad \dots\dots(2),$$

give the values in Table X.

Table X.

Acetic and butyric acids actually added equalled 81.0 and 64.6 c.c. respectively of 1.042 *N*/50 alkali;  $d_1$  and  $d_2$  were taken to be 30.6 and 69.7 respectively.

Alkali (1.042 *N*/50) required for neutralisation

Name of soil	Acetic acid recovered c.c.	Error %	Butyric acid recovered c.c.	Error %
Central Farm, Coimbatore—garden soil ...	82.5	+1.9	63.8	-1.2
Rothamsted, Barn Field—dunged plot ...	80.3	-0.9	64.6	0.0
Anakapalli, Madras—paddy ...	82.3	+1.6	64.4	+0.3
Yessgaon, Bombay ...	79.6	-1.7	65.3	+1.1
Punjab—wheat ...	80.7	-0.4	64.7	+0.2
Mandalay, Burma—paddy ...	80.5	-0.6	65.4	+1.2
Probable error of a single determination ...	±0.9 %		±0.6 %	

It has to be inferred that (a) the extraction by leaching was quantitative and (b) the technique adopted led to accurate determination of the acids.

*Effect of mass of soil and time of standing.*

Further trials (Table XI) showed that the results were not affected by variations in the amount of soil or the time of standing in contact with sterile soil.

Table XI.

Paddy soil from Anakapalli, Madras, was used. Acetic and butyric acids added equalled 73.6 and 107.4 c.c. of 1.042 N/50 alkali respectively.

Acids recovered in terms of 1.042 N/50 alkali.

Weight of soil	Acetic acid	Butyric acid	Time of standing with 25 gm. of soil	Acetic acid	Butyric acid
15 gm.	73.2 c.c.	108.0 c.c.	4 hours	72.8 c.c.	106.8 c.c.
50	74.0	107.2	8	73.8	106.2
75	73.8	107.0	24	72.6	106.6
100	74.2	106.8	—	—	—
Probable error of a single determination }	$\pm 0.4$ %	$\pm 0.3$ %		$\pm 0.7$ %	$\pm 0.6$ %

Distilling constants for propionic and isobutyric acids at low concentrations were found to be  $52.5 \pm 0.2$  and  $79.6 \pm 0.3$  respectively.

Soils are but complex biological media and the foregoing methods which were applicable to a variety of soils can, with greater facility, be extended to study changes in other types of biological media as well.

SUMMARY.

Methods for extraction, concentration and determination of minute quantities of soluble carbohydrates, lactic acid and volatile fatty acids have been described. Different factors affecting the accuracy of the determinations have been studied and corrections, where necessary, have been suggested.

The author's thanks are due to Mr H. J. Page for his encouragement and suggestive criticism.

REFERENCES.

- (1) ALLEN, A. H. *Commercial Organic Analysis*, 5th ed. (1924).
- (2) DYER, D. C. *J. Biol. Chem.* (1917), **28**, 445.
- (3) LANE, J. H. and EYNON, L. *J.S.C.I.* (1923), **42**, 32 T.
- (4) PAESSLER, J. *Chem. Zentr.* (1908), **1**, 66.
- (5) SUBRAHMANYAN, V. *J. Agric. Sci.* (1929), **19**, 627.
- (6) SUTTON, F. *Volumetric Analysis*, 11th ed.
- (7) VAN SLYKE, D. *J. Biol. Chem.* (1917), **32**, 455.

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*The Life-Cycle of the Nodule Organism, Bacillus Radicicola (BEIJ.), in Soil and its Relation to the Infection of the Host Plant.*

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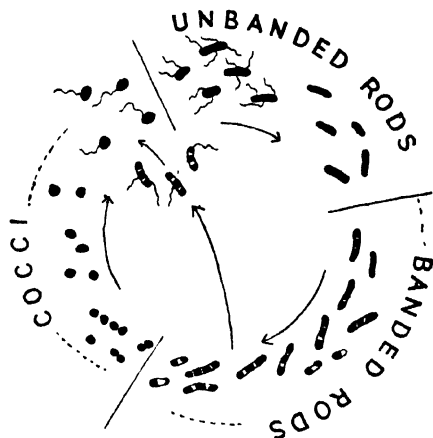
(Communicated by Sir John Russell, F.R.S.—Received January 7, 1926.)

A. INTRODUCTION.

The existence of changes in the form of *Bacillus radicicola* has been known since Beijerinck (2) first isolated it in 1888 from leguminous plant nodules. He observed the motile "swarmer" stage as well as the branching forms, whose nature was already the subject of controversy. About the same time the development of straight-rod forms of the organism was described by Prazmowski (14). Numerous writers have since observed the existence of the organism in the three conditions of straight rods, branching rods and cocci (for references, see Löhnis, 1921 (10)). In 1916 Löhnis and Smith (11) claimed that the various forms constituted a definite life-cycle through which the organism normally passes, and this cycle, as seen in cultures, was carefully described in 1919 by Bewley and Hutchinson (3). In a vigorous young culture, the predominating form of the organism is a short, evenly staining rod (fig. 1). These rods soon undergo a change in internal structure, the staining material becoming segregated into bands crossing the cell. During this banded stage the cells frequently become swollen, distorted, and branched, the so-called "bacteroids" (Brunchorst (4)), but this irregularity of form is not an essential part of the life-cycle, but would appear to be a response to conditions of the environment (Buchanan, 1909) (5). The banded cells give rise to the cocci by further condensation of the bands.

The origin of the cocci within the mother-cell was described and illustrated in 1891 by Morck (12), who was the first to appreciate the relation of the internal structure of the cell to the life-history of the organism. The cocci are usually released in a non-motile condition, and afterwards develop flagella, becoming actively motile, the "swarmers" of Beijerinck (2). Under certain conditions, however, the cocci develop flagella while still enclosed within the mother-cell. This condition has been described by Greig-Smith (8) and the observation confirmed by one of the present authors (7). The cocci eventually become elongated and thus pass into the unbanded rod stage. The flagella, which are

developed on the cocci, persist after this elongation, but are soon lost: the rods then become non-motile. The development of motility in a culture is thus intimately associated with the appearance of the coccus stage.



THE LIFE CYCLE OF *BACILLUS RADICOLA*

FIG. 1.

In 1922 Wallin (15) observed the existence of various stages of the organism within the tissues of the nodule. The object of the present work was to discover whether the nodule organism undergoes a similar cycle of changes in soil, and, if so, whether the incidence of the motile coccus stage is associated with a migration of the bacteria through the soil.

## B. TECHNIQUE.

### (1) *Method of studying the Life-Cycle in Soil.*

The nodule organism that infects lucerne (*Medicago sativa* L.) was used throughout the work. The strain was originally supplied by the Statens Planteavlslaboratorium, Lyngby, Copenhagen, and its ability to produce nodules was frequently tested. The cultures were grown on an agar medium containing mineral salts, saccharose, and extract of lucerne roots, and were incubated at 25° C. for 10 days before use.

Petri dishes, 6 inches in diameter, containing 200 grs. of a mixture of Rothamsted soil (34 per cent.) and sand (66 per cent.) were sterilised at 15 pounds pressure for 30 minutes, and the water content was made up to

17 to 18 per cent. on an air-dry basis. An agar slope culture was filled to the top of the slope with sterile inoculating fluid, the bacterial growth scraped off with a sterile platinum loop and, when the sediment had settled, 1 c.c. of the suspension was removed in a sterile pipette and evenly distributed over the petri dish of soil. The soil was then thoroughly mixed with a sterile spatula and incubated at 25° C.

On each occasion of sampling three cores were removed from different parts of the petri dish by means of a sterile cork borer. These were well mixed in a watch-glass containing 1 c.c. of sterile distilled water, the sample was triturated with a small sterile rubber pestle and, after standing for 10 minutes, two loopfuls of the surface fluid were spread over a clean slide and the film dried at 45° C. in an oven.

In staining the films a modification of Winogradsky's method (16) was used. The dried film was fixed with absolute alcohol and flooded with phenol-erythrosine (1 gram of erythrosine dissolved in 100 c.c. of 5 per cent. phenol), which was allowed to act for 10 minutes. After washing in tap water the film was re-stained with 2.5 per cent. aqueous erythrosine for another 10 minutes. Duplicate preparations were made from each sample, and, from these, the percentages of unbanded rods, banded rods, and cocci were determined for each slide by counting the numbers of each stage in five random microscope fields.

(2) *Test of Accuracy of the Sampling and Microtechnique.*

Table I.\*—Showing the Observed and Expected Numbers of Bacterial Forms.

Samples.	Total number of cells counted.	Number of cocci.		Number of unbanded rods.		Number of banded rods.	
		Observed.	Expected.	Observed.	Expected.	Observed.	Expected.
A .....	277	13	13.66	257	255.36	7	7.98
B .....	337	18	16.61	308	310.67	11	9.72
C .....	290	16	14.30	267	267.34	7	8.36
D .....	171	6	8.43	159	157.64	6	4.93
Mean percentage .....		4.93	—	92.19	—	2.88	—
Percentage standard error .....		0.66	—	0.82	—	0.51	—

\* The authors are indebted to Mr. R. A. Fisher for his advice, and, in particular, for making a statistical test of the data given in Table I.

The accuracy of the sampling and microtechnique was tested in four simultaneous samples, taken during the course of experiment 1. From the counts thence obtained the mean percentage of each cell-type was calculated for the whole set of samples. The expected numbers of each type in the individual samples were then obtained by taking the mean percentage of the total cells counted in that sample. Table I shows the expected numbers with the actual numbers observed. The variation between individual samples is not greater than necessarily follows from the random distribution of organisms in the soil.

### C. LIFE OF THE ORGANISM IN STERILE SOIL.

#### *Experiment I.*

In the preliminary study of the organism growing in sterile soil, a suspension in distilled water was used to inoculate the soil as described above. Samples of soil were examined immediately after inoculation, and, thereafter, at hourly intervals for 27 hours and then at daily intervals for six days.

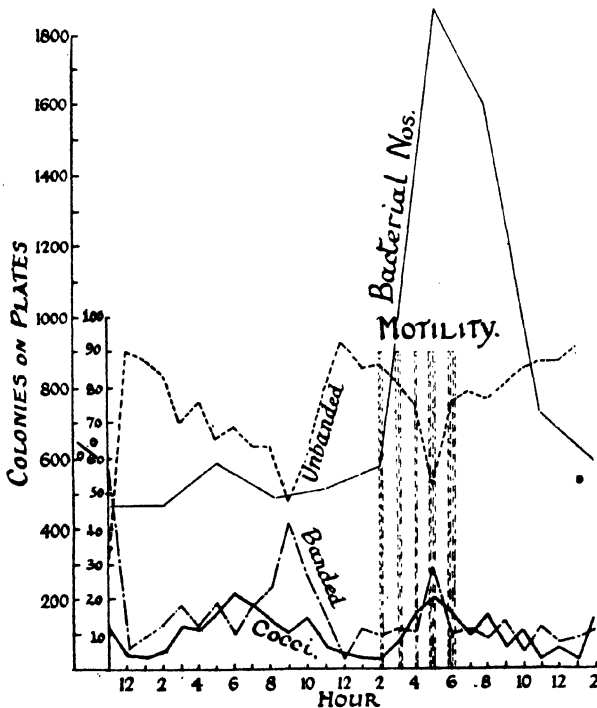


FIG. 2.—*Bacillus radicum*. Life-cycle changes in sterile soil.

(1) *The Life-Cycle Changes.*

In any sample examined, all the three principal cell-types occur, but their proportions vary at different times. The study of the life-cycle is therefore a quantitative problem, it being necessary to follow the changes in the relative numbers of these stages. The changing percentages of each cell-type during the first 27 hours is illustrated in fig. 2. Within this period the population passed through two complete cycles of changes, each commencing with an increase in the proportion of unbanded rods and followed by a rise in the proportion of cocci and banded rods. In both cycles the maximum percentage of cocci is reached as soon as, or before, that of the banded rods from which they arise. This feature is also seen in later experiments (figs. 3, 4, 5, 6, 7), and may be due to a change in the relative rates at which cocci are formed and at which they are released from the rods. The regularity of the changes here observed was shown in a second soil culture set up in a similar manner, from which samples were examined every two hours for 14 hours. The percentages of each cell-type found in this culture are plotted in fig. 3, together with the percentages found at equivalent times in experiment 1.

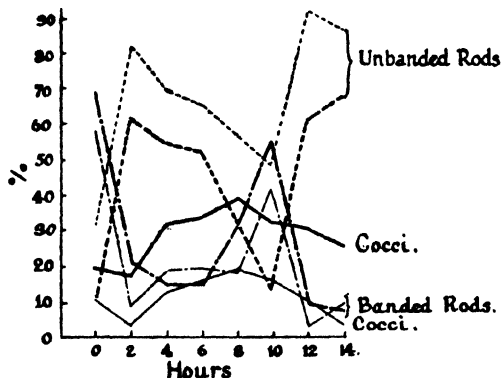


FIG. 3.—Life-cycle changes found in two experiments.

(2) *Multiplication.*

*Bacillus radicicola* multiplies in two ways, by binary fission of the rod stage and by multiple fission, the banded rods breaking up into cocci. Plate counts made during the course of the experiment indicate that the latter method is the more important, for during periods of maxima of the unbanded rods the numbers show no increase, but increases occur on both occasions when the cocci reach their maxima.

### (3) *Motility.*

To study the relation between the life-cycle and the occurrence of motile forms in the soil, a drop of soil suspension was examined in a fresh state at each time of sampling and the presence of motile forms was noted. The vertical broken lines in fig. 2 show the times when motile cells were observed. Motile forms were not evident within the first 12 hours, perhaps owing to the small total number of cells in the culture. The greatest number of motile cells was found in the samples taken at 5 a.m., when the cocci had reached their second maximum, and at 6 a.m., when the relative numbers of cocci were falling off and being replaced by unbanded rods. This is in accordance with the fact observed in liquid cultures, that flagella are developed on the cocci and persist for some time after these lengthen to form the unbanded rods.

### (4) *Later Course of the Life-Cycle.*

The subsequent history of the soil culture was followed in experiment 1 by taking samples at daily intervals for six days, and the percentages of each cell-type during this period are shown in fig. 4, where also the earlier history of the culture, already described, is summarised by plotting points selected at six-hourly intervals. In the first 24 hours the cocci never exceed 25 per cent. of the population, but a big increase subsequently occurs, so that by the third day about 70 per cent. of the cells are in this stage.

The chief features of the life-cycle during the first six days were confirmed by a separate experiment carried out under conditions similar to experiment 1, during which samples were examined at daily intervals. In fig. 4 the results of the two experiments are plotted together. The fact that the changes in relative numbers of each cell type, both at hourly intervals during the first 12 hours (fig. 3) and at daily intervals during six days (fig. 4), are so closely reproduced in entirely separate experiments shows that a regular cycle occurs in the soil, the principal features of which are constant in a given environment. It was important to determine whether this cycle was inherent and unalterable, or whether it could be modified by changes in the environment.

## D. INFLUENCE OF THE INOCULATING FLUID IN MODIFYING THE LIFE-CYCLE.

Bewley and Hutchinson (3) observed that in cultures of *B. radicola* a plentiful supply of calcium phosphate in the medium stimulated the appearance of the motile coccus stage. To see whether the nature of the suspending fluid used in inoculating soil influenced the life-cycle in the soil, experiments were

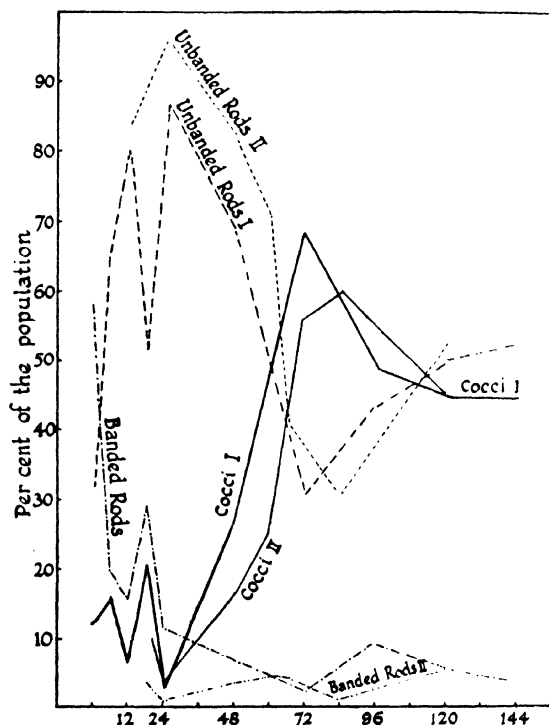


FIG. 4.—Later course of the Life-cycle of *Bacillus radicicola* in soil inoculated with a suspension in water. (Two experiments.)

conducted in a manner similar to experiment 1, save that the following fluids were used to make the inoculating suspension :—

A. Milk.

B. 0·1 per cent. solution of di-acid calcium phosphate ( $\text{CaH}_4(\text{PO}_4)_2 + 4\text{H}_2\text{O}$ ) in water.

C. 0·1 per cent. solution of di-acid calcium phosphate in milk.

Samples of the soil cultures were examined at daily intervals for six days. The changing percentages of cocci, banded and unbanded rods, are shown in figs. 5, 6 and 7, which should be compared with experiment 1 (fig. 4), where the inoculum was suspended in distilled water. In all these experiments a high percentage of unbanded rods at the twenty-fourth hour gives place to a rise, first in the proportion of cocci, and secondly in that of banded rods. The nature of the inoculating fluid, however, strikingly affects the time and degree of coccus formation (fig. 8).

With distilled water as the inoculating fluid, the percentage of cocci increases but slowly for 48 hours and reaches a maximum at the third day, then falling

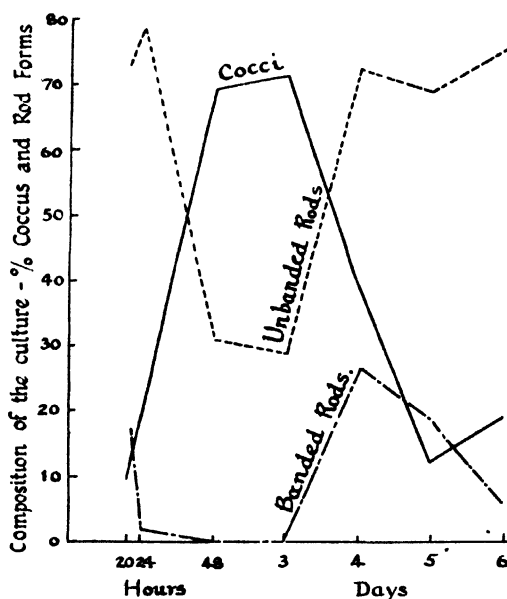


FIG. 5.—Changes in the life-cycle of *Bacillus radiculicola* in soil inoculated with a suspension in water + calcium phosphate.

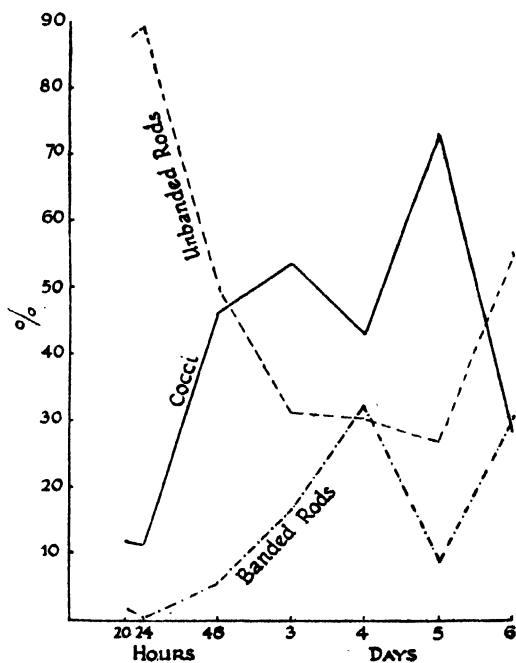


FIG. 6.—Changes in the life-cycle of *Bacillus radiculicola* in soil inoculated with a suspension in milk.

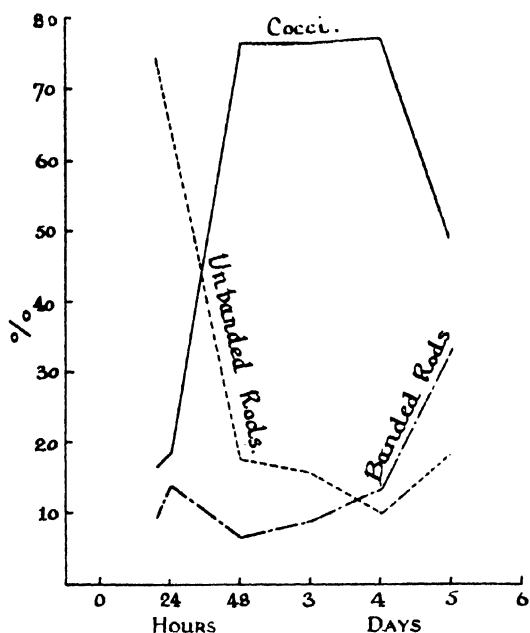


FIG. 7.—Changes in the life-cycle of *Bacillus radicicola* in soil inoculated with a suspension in milk + calcium phosphate.

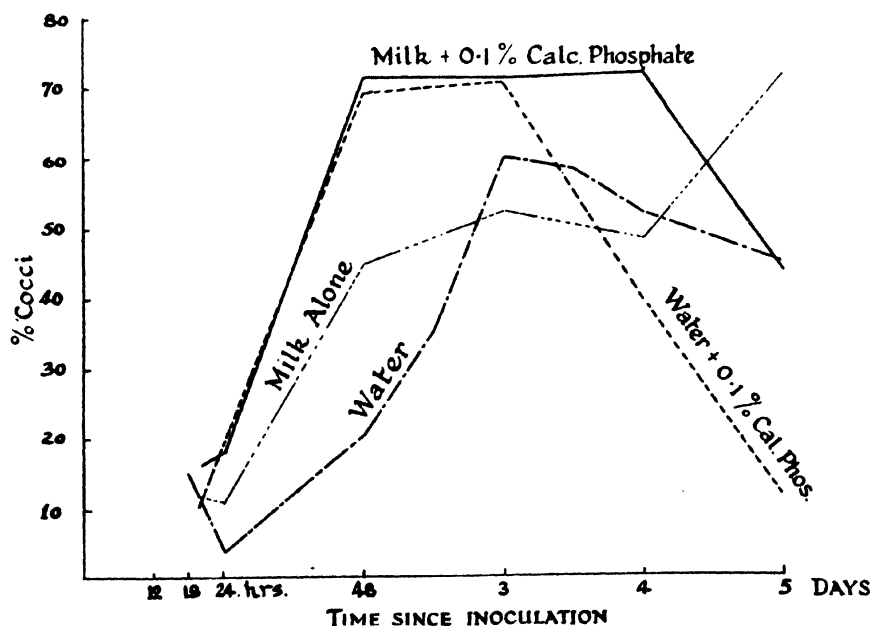


FIG. 8.—Effect of the inoculating fluid on the appearance of the cocous stage in soil.

off. The addition of calcium phosphate to the inoculating fluid, whether this be milk or water, causes a rapid preponderance of cocci, which, within 48 hours, form about 70 per cent. of the population. The use of a milk inoculum lengthens the time during which the cocci predominate.

Since a rise in the percentage of cocci coincides with the appearance of motile forms in the soil, the different inoculating fluids, which modify the time of coccus predominance, should also influence the time of appearance of the motile cells in the culture. To determine how soon motile forms appeared when the bacterial suspensions in the various fluids used above were inoculated into soil, suspensions of a 10-days' old agar culture of *Bacillus radicola* were made in these fluids. These suspensions were used to inoculate test-tubes containing 25 grams of sterile soil and sand mixture. Hourly examinations were made, fresh preparations being searched for motile organisms. The times when motile forms were first observed are recorded in Table II.

Table II.—Hour of Appearance of Motile Forms (from the Time of Inoculation).

*Inoculum.*

Tap water .....	Between 18th–20th hour.
Skim milk .....	Between 15th–18th hour.
0.1 per cent. calcium phosphate in water .....	Between 18th–20th hour.
Skim milk + 0.1 per cent. calcium phosphate.....	Between 10th–12th hour.

Cultures added to the soil in suspensions, either in water or in a solution of phosphate in water, take the same time to produce motile forms in the soil. A milk suspension, however, produces motile forms earlier, and the addition of the phosphate to the milk greatly shortens the time of their appearance as compared with milk alone.

#### E. SPREAD OF THE BACTERIA THROUGH STERILE SOIL.

If the bacteria move actively through the soil, the nature of the inoculating fluid, which alters the time of appearance of the motile coccus stage, should similarly affect the time of commencement of spread from the point of inoculation.

Previous workers differ as to the importance of active motility in influencing the spread of *Bacillus radicola* through soil. Ball (1909) (1) found that, in suitably moist soil, the bacteria diffused at a rate of 1 inch in 48 hours. He concluded that movement was largely due to soil-water currents aided by multiplication and motility of the organism. Kellerman and Faucett (9) found that in saturated soil, at 25° C., the bacteria moved at a rate of 1 inch in 48 hours. In barely moist soil, or at 10° C., this rate was reduced to about 1 inch in 72 hours. They assumed, however, that the movement was due to

the multiplication of the bacteria. Frazier and Fred (1922) (6), however, concluded as a result of pot and field experiments, that soy-bean nodule bacteria spread very slowly, if at all, unless passively carried by water currents.

In the present work, the spread from the centre of petri dishes of soil was studied as described in the footnote.\* The bacteria spread evenly in each direction (fig. 9). Their migration was, therefore, little affected by inequalities of soil conditions in the petri dish.

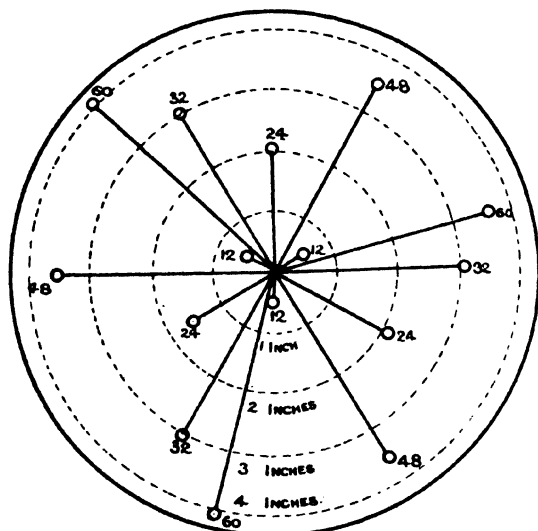


FIG. 9.—Radial spread of *Bacillus radicicola* from the centre of a petri dish of soil. The straight lines show the distances reached in each direction in the number of hours indicated.

\* Petri dishes  $8\frac{1}{2}$  in. in diameter were filled each with 400 grams of the soil and sand mixture as used in experiment 1, and the moisture content brought up to 18 per cent. The lid of the petri dish was lined with filter paper to prevent condensation water from dripping off the lid on to the soil. The petri dishes were sterilised in the autoclave. A cork-borerful of soil-sand mixture was removed from the centre of each petri dish, and the space thus left was filled with air-dry soil and sand, previously sterilised. Upon this was placed one drop of bacterial suspension made up in the inoculating fluid to be tested. The small column of air-dry soil-sand mixture absorbed the drop and prevented a local accumulation of moisture, which might have led to water currents carrying the bacteria outward. The dishes were incubated at  $22^{\circ}\text{C}$ ., and, at intervals, samples were taken with a sterile cork borer every  $\frac{1}{4}$  in. along three radii. From each sample preparations were made, following the technique used for the life-cycle studies, and a loopful of each sample was streaked upon a slope of lucerne root-extract agar. The presence or absence of the organism at given distances from the centre along three different radii was then determined by examination of stained preparations, and the observation confirmed by the presence or absence of growth on agar slopes. There was a close agreement between the results of these two methods.

Fig. 10 shows the results of a number of trials in which bacterial suspensions in water alone, in milk alone, and in milk + 0.1 per cent.  $\text{CaH}_2(\text{PO}_4)_2 + 2\text{H}_2\text{O}$  were used to inoculate the centre of the dish. The trials were made in duplicate, using water and milk suspensions, and in triplicate, using milk and phosphate, the curve on the left of the figure representing two trials, the results of which were identical. The nature of the inoculating fluid greatly influenced the time at which spreading commenced.

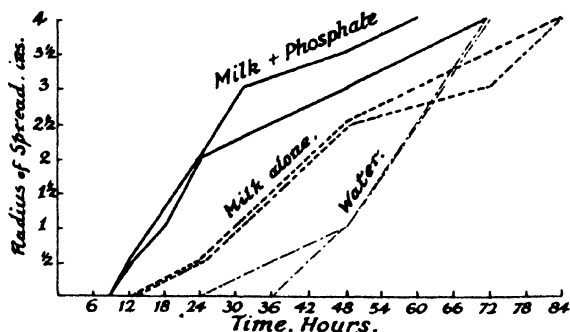


FIG. 10.—Influence of the inoculating fluid on the spread of *Bacillus radicola* through soil.

In fig. 11 the influence of the three inoculating fluids on the spreading is compared with their influence on the percentage of cocci found in soil at the same intervals of time during the life-cycle studies. The inoculating fluid similarly affects the time of coccus formation and the time at which spreading commences, suggesting that the motile cocci actively migrate through the soil.

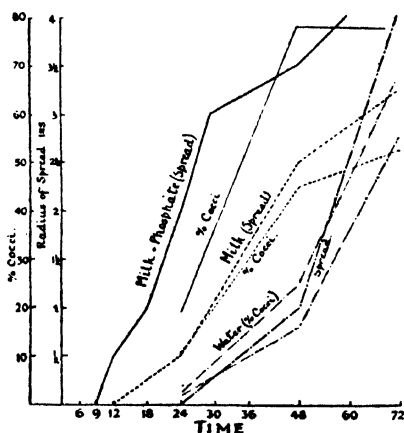


FIG. 11.—Influence of the inoculating fluid on the spread of *Bacillus radicola* and on the percentage of cocci formed in soil.

In this experiment, the organisms, in the course of spreading, must soon have removed themselves from the direct influence of the inoculating fluid, since the inoculum was added only to the centre of the dish. A more rapid spread, however, by distributing the population through a larger volume of soil, would tend to increase the available food supply and might thus exert a comparatively remote influence on the multiplication of the organisms. By the use of the technique described in the footnote,\* the effect of the inoculating fluid on bacterial numbers was studied.

Fig. 12 shows the distribution of bacterial numbers in troughs of soil and sand mixture inoculated at one end with a bacterial suspension in water. After 24 hours the organisms are concentrated near the point of inoculation, the soil beyond the second inch being sterile. By the fourth day little increase in total bacterial numbers is found, but the bacteria are now dis-

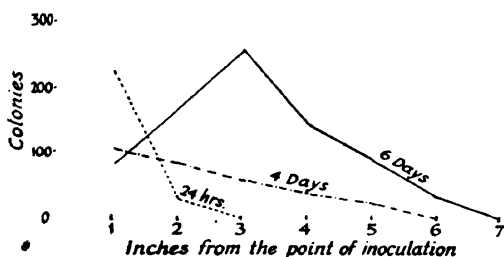


FIG. 12.—Numbers of *Bacillus radicicola* in soil at varying distances from the point of inoculation. Inoculum suspended in water.

\* 1500 grams of soil-sand mixture were placed in a "Pyrex" glass trough 3 in. deep, measuring 10 in. by 4½ in. at the top, and having a floor measuring 8 in. by 3 in. The moisture content of the mixture was made up to 18 to 19 per cent. A cork-borerful of soil was removed from the end of the trough and replaced by small beads. Care was taken to pack the beads closely so that they were in contact with the surrounding soil. A ridged zinc cover, having a hole at each end of the ridge, and with the sides projecting downwards 1 in. all round, was made to fit over the glass trough. The holes and the space between the projecting metal sides and the glass trough were packed with cotton-wool, and the apparatus was sterilised at 15 lb. pressure for half an hour.

The bacterial suspensions were prepared with the three inoculating fluids—(1) water alone, (2) skim milk alone, and (3) skim milk + 0.5 per cent. di-acid calcium phosphate. The glass beads were just moistened with the suspension to be tested, and the trough then incubated at 22° C.

For each inoculating fluid three troughs were used, and bacterial counts were made at intervals of one, four and six days, one trough being opened on each occasion. Duplicate samples were taken 1 in. away from the point of inoculation, and at intervals of 1 in. up to the eighth inch, and plate counts of the bacteria were made from each sample. A close agreement between the duplicate samples was always found.

tributed as far as the fifth inch. By the sixth day the organisms have reached the sixth inch, and a considerable increase in numbers has taken place. As compared with the numbers after four days, there has been an increase of about four-fold at the third, fourth and fifth inches, but at the second inch the numbers are less than doubled, while at the first inch there has been an actual decrease in numbers. This distribution of numbers indicates that the organisms are better able to multiply in the recently infected soil than near the point of inoculation, where their previous growth has rendered the soil a less favourable medium. Actual migration of motile forms may be a contributory cause.

Fig. 13 shows the distribution of numbers in troughs inoculated with a suspension in milk. Here, again, a multiplication near the point of inoculation occurs in the first 24 hours, and subsequently spreading occurs, the bacteria reaching the fifth inch on the fourth day and the sixth inch on the sixth day.

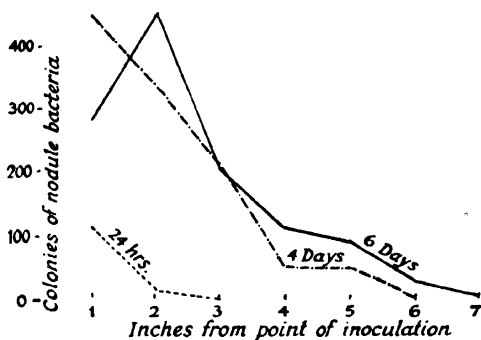


FIG. 13.—Numbers of *Bacillus radicicola* in soil at varying distances from the point of inoculation. Inoculum suspended in milk.

The milk, however, produces a great effect in stimulating multiplication as compared with the water inoculum. This stimulation is, however, confined to the region neighbouring the point of inoculation, the numbers found beyond the second inch on the sixth day being no higher than was the case with the water inoculum.

Fig. 14 shows the distribution of numbers in troughs inoculated with a suspension in milk + 0.5 per cent.  $\text{CaH}_2 + (\text{PO}_4)_2 \cdot 2\text{H}_2\text{O}$ . The addition of the phosphate causes a marked increase in multiplication, and also in spreading, the bacteria reaching the farther end of the trough within four days. There is a considerable fall in numbers at the first and second inch on the fourth and sixth days, and this is perhaps due to the high numbers in that region after 24 hours.

The addition of the phosphate, however, produces a very great increase in numbers from the third to the fifth inch. By the sixth day the region of highest numbers has moved forward to the fourth inch, where the numbers are more than three times as great as with the milk inoculum without phosphate. In considering what is the cause of this increase, the small amount of phosphate added to the trough must be recognised. About 0.25 c.c. of the bacterial suspension in milk + calcium phosphate was used to inoculate the end of each trough. The 1.25 mgs. of phosphate contained in this can scarcely have exerted an appreciable direct action on the number of organisms four and five inches from the point of inoculation. The curves, however, show that the bacteria increase most rapidly in the newly infected soil, so that the known effect of the phosphate

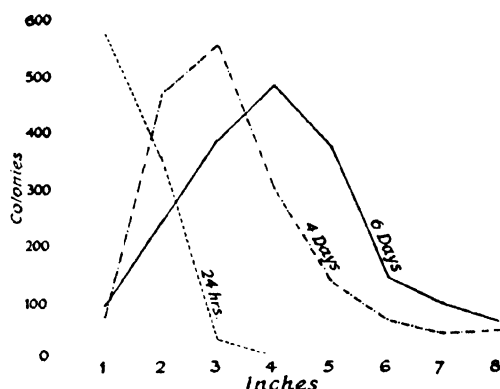


FIG. 14.—Numbers of *Bacillus radicicola* in soil at varying distances from the point of inoculation. Inoculum suspended in milk + calcium phosphate.

in hastening migration of the bacteria through the soil supplies an explanation of its remote influence on the multiplication. The invasion of fresh soil by motile individuals is followed by a wave of high bacterial numbers, due to multiplication of the bacteria in the freshly invaded soil. The action of the phosphate in stimulating the spreading consequently results in an extension of the wave of high numbers to a greater distance from the inoculation point in a given time. The addition of calcium phosphate to the milk used as inoculating fluid thus produces a two-fold effect on the subsequent distribution of the bacteria in the surrounding soil:—first, it enlarges the volume of soil infected in a given time, and, secondly, it increases the number of organisms within that volume. In the practice of seed inoculation, both these factors should increase the chances of root-infection, and consequently favour the formation of nodules.

## F. INFLUENCE OF THE INOCULATING FLUID ON NODULE FORMATION.

A series of pot experiments were made to see whether the addition of calcium phosphate to the inoculating fluid would increase the formation of nodules.

*Pot Experiment 1.*

Thirty cylindrical glazed earthenware pots,  $6\frac{1}{2}$  inches in diameter and 15 inches deep, were each filled with 22.5 lb. of sieved garden soil, to which 30 per cent. sand had been added. The moisture content of this mixture was made up to about 20 per cent. before filling the pots. The soil used was one in which lucerne nodule bacteria were present in small numbers, and in order to test the additional effect of the inoculation in such soil, neither soil nor seed were sterilised before use. Thick suspensions of the bacteria were made in skim milk alone, and in skim milk to which 0.1 per cent. di-acid calcium phosphate had been added. Uniform weights of seed were wetted with equal volumes of each suspension and the seed sown at the rate of 1 gram per pot. Ten parallel pots with each type of inoculation were set up and ten pots were sown with uninoculated seed. All pots received equal watering and the plants were thinned out in successive stages, so that at the sixteenth week, five plants per pot remained. Nodule counts were made after 4, 5, 6, and 16 weeks. For this purpose the roots were washed with a fine jet of water from a hose. In counting the nodules, the roots were floated in a shallow dish and examined with a lens against a black surface. Dry weights of crop were also taken.

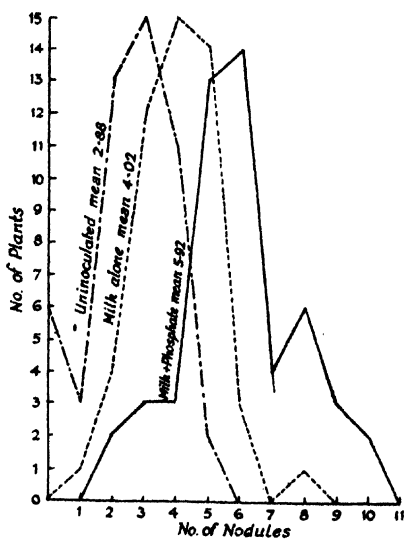


FIG. 15.

The distribution of nodules on individual plants at the fourth, fifth, and sixteenth weeks is shown in the form of frequency curves in figs. 15, 16, and 17. The effect of the phosphate is increasingly evident with the age of the plant,

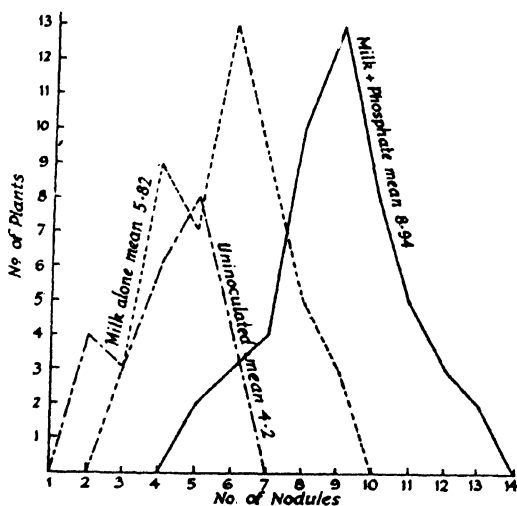


FIG. 16.

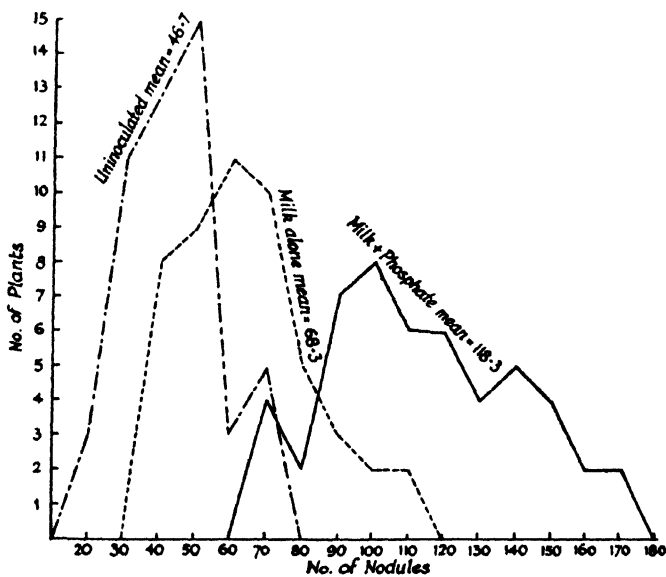


FIG. 17.

but it is undoubtedly significant even at the fourth week. There has also been an effect on the weight of the plants, as is seen in Table IV.

Table IV.—Showing the Yield of Lucerne at the Sixteenth Week.

Treatment.	Mean number of nodules.	Mean dry weight.		Nodules per gram of roots.
		Tops.	Roots.	
Uninoculated .....	47·3	9·05	12·67	3·8
Milk inoculum .....	65·2	10·72	13·66	4·7
Milk 0·1 per cent. calcium phosphate inoculum ....	116·2	12·71	14·42	8·07

*Pot Experiment II.*

In this experiment the soil and sand mixture was steamed for eight hours, and sterilised skim milk was used in making up the inoculating suspensions, but the seed was not sterilised. An additional series of pots was set up, sown with seed inoculated with a suspension of bacteria in 0·1 per cent. solution of di-acid calcium phosphate in distilled water. Eight parallel pots of each treatment and six pots of uninoculated seed were sown, each pot receiving 2 grams of seed and the seedlings being thinned out as before. Nodules were counted on the second, third, fourth, fifth and sixteenth weeks, and the results are shown in Table V.

Table V.—Showing the Mean Number of Nodules per Plant.

Age of plant.	No. of plants.	Uninoculated.	0·1 per cent. phosphate inoculum.	Milk inoculum.	Milk and 0·1 per cent. phosphate inoculum.
2nd week ....	50	2·04	3·00	3·38	3·28
3rd week .....	50	2·42	3·20	3·70	3·67
4th week .....	50	1·90	3·86	3·78	4·18
7th week .....	20	10·65	19·6	15·45	15·9
16th week ....	10	43·8	56·2	59·0	113·1

The addition of phosphate to the milk has again caused a big increase on nodule numbers by the sixteenth week (see fig. 18), though there is no significant effect before this.

The plants were harvested and dry weights taken at the sixteenth week, but five pots of each treated series and three of the uninoculated were left after cutting the crop, and the second growth after 30 weeks was cut and weighed. The effect of the milk phosphate on the growth is more marked at the thirtieth week than after 16 weeks (Table VI).

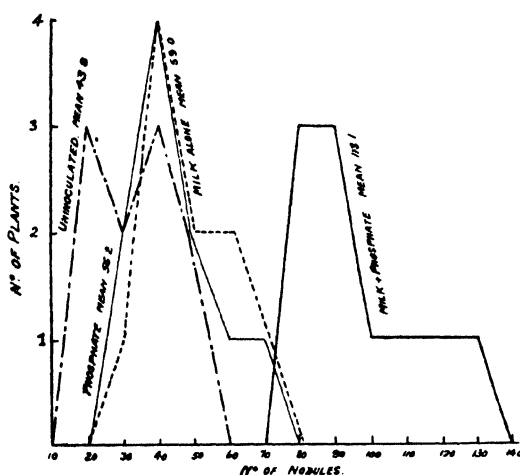


FIG. 18.

Table VI.—Pot Culture Trial of Lucerne Inoculation.

Treatment.	Result after 16 weeks.		Result after 30 weeks.	
	Mean No. of nodules.	Dry weight of tops.	Dry weight of tops.	Dry weight of roots.
Uninoculated .....	43.8	12.6	9.2	11.5
Inoc. milk suspension .....	59.0	12.1	9.8	12.1
Inoc. suspension in aqueous salt calc. phos. 0.1 per cent. ....	56.2	13.9	9.9	12.8
Inoc. suspension in milk 0.1 per cent. calc. phos. ....	113.1	14.4	12.6	15.5

### *Pot Experiment III.*

In this experiment, 25 pots were used, each containing 22.5 lb. of soil-sand mixture.

The series was divided into the following sets :—

**Set I. Inoculated with bacterial suspension in skim milk alone.**

II.       "       "       "       "       "       "       +0.1 per cent.

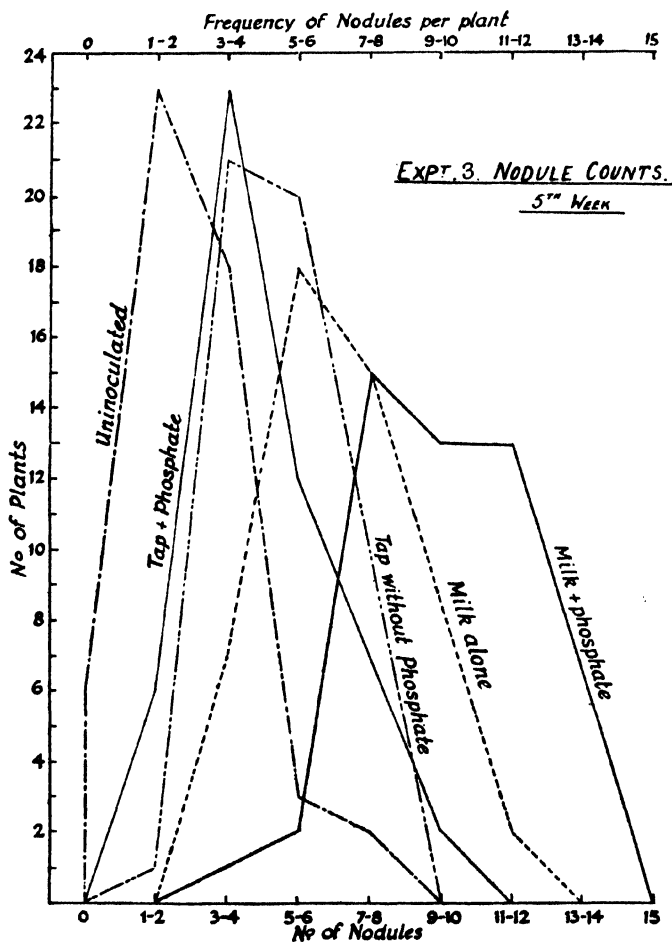
$$\text{CaH}_4(\text{PO}_4)_2 + 2\text{H}_2\text{O}$$

„ III. „ „ „ „ in tap water + 0.1 CaH<sub>4</sub>(PO<sub>4</sub>)<sub>3</sub>

$$2\text{H}_2\text{O}$$

„ IV. „ „ „ in tap water alone.

„ V. Uninoculated.



**FIG. 19.**

The pots and soil were sterilised by steaming and each pot received 1 gram of seed, which, in the inoculated sets, was wetted with a counted suspension of

bacteria at the rate of 13,600 bacteria per seed. The pots were watered at intervals with boiled water. Fig. 19 shows the numbers of nodules which developed after five weeks.

This experiment confirms the effect of adding di-acid calcium phosphate to milk in increasing nodule development. The addition of the phosphate, however, has in no way improved tap water as an inoculating fluid.

#### G. DISCUSSION.

The pot trials above described show the increased nodule formation from seed inoculated with a bacterial suspension in milk + di-acid calcium phosphate over that inoculated with a suspension in milk alone. The dose of phosphate that produces this effect is minute. Approximately 0.2 c.c. of the bacterial suspension was used to wet 1 gram of seed, and this volume contained 0.1 per cent. of calcium phosphate. The amount of calcium phosphate added to each pot containing about 20 lb. of soil was therefore about 0.2 mg. in pot experiments I and III and 0.4 mg. in experiment II. So small a dose can scarcely have had any direct influence on the plant growth. It has been shown, however, that doses of this order or magnitude, by affecting the production of motile forms of the bacteria, do influence their migration through the soil and exert a remote influence on the bacterial numbers. The increased nodule formation can therefore be attributed to the wider distribution of the bacteria and to their greater numbers at a distance from the seed.

If this explanation is correct, the added calcium phosphate should show its effect in increasing the nodule numbers towards the extremities of the roots rather than near the seed. In order to test this point the following experiment was carried out:—32 glazed earthenware pots were filled with soil and sand mixture. Half of them were sown with lucerne seed inoculated with a bacterial suspension in milk + 0.1 per cent.  $\text{CaH}_4(\text{PO}_4)_2 + 2\text{H}_2\text{O}$  and half with seed inoculated with a suspension in milk alone. A standardised inoculum was used, so that each seed received approximately 4,500 bacteria. 0.25 gram of seed was sown per pot and the plants thinned out as seemed necessary. At intervals during the growth period duplicate pots of each type were removed, the roots washed and separate counts of the nodules in the top 4 inches of the root and below that depth were made. The development of nodules in the surface region and in the deep region is shown in fig. 20.

The addition of calcium phosphate produces no increase in nodules in the top four inches of the root, near the point of inoculation. There is consequently

no effect from the phosphate until the sixth week, when the root system begins to develop below this depth. From this time onwards the phosphate produces an increase in nodule numbers, which, however, is entirely confined to the distal region of the root system. The effect of the phosphate consequently becomes more marked as the plant grows older and the lower roots develop.\*

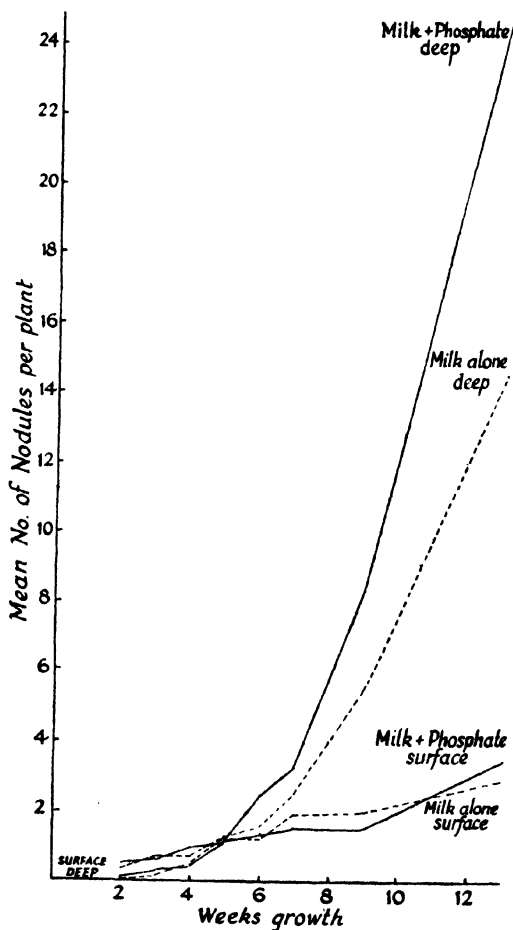


FIG. 20.—Influence of the inoculating fluid on the number of nodules produced near the surface and in the deeper parts of the root system.

This increasing effect of the phosphate with the age of the plant is also seen in pot experiments I and II (fig. 21).

\* The authors are gratefully indebted to Mr. P. H. H. Gray for his assistance in making counts of the nodules in this and in the other pot experiments.

While the effects produced by phosphate are thus in accordance with the above explanation, they also show that the spread of the organisms in the soil of the pots cannot proceed and continue at the rate recorded in the laboratory experiments. The rate of radial migration in petri dishes was found to be of the order of one inch in 24 hours, while in the glass-trough experiments the wave of high bacterial numbers appears to move along the trough at an approximate rate of one inch in 48 hours. While the experimental data on this point are still insufficient, they indicate a rate of migration which, if maintained in the pot trials, would result in the infection of the whole mass of soil in about 30 days, even with the least efficient method of inoculation. Measurements of spreading have not, however, been carried out for long enough period to decide to what

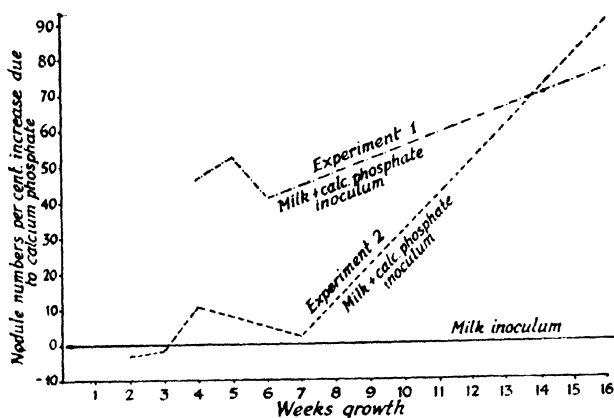


FIG. 21.

extent it is maintained, and the pot experiments suggest either that the initial rate of spread is not maintained or else that, under the different soil conditions in the pot, it occurs at a sufficiently slow rate to enable the addition of phosphate to produce an effective increase in bacterial numbers in the lower layers of soil even later than six weeks after sowing.

The effect of calcium phosphate, as shown in the above experiments, has a bearing on the commercial methods of seed inoculation. The idea of inoculating seed with pure cultures of the nodule bacteria was first conceived by Nobbe (13) in 1896 in collaboration with Hiltner. The method of seed inoculation has since been tested extensively with varying success in many parts of the world, and in these trials a number of different liquids have been used in making the bacterial suspension with which the seed was wetted. In 1902 the use of a suspension of the bacteria in skim milk as the inoculum was tried, and this method is now

used extensively and with success in Denmark for the inoculation of lucerne seed. The addition of di-acid calcium phosphate to the milk is thus a simple modification of an already existing practice, and the cost of it should be negligible on account of the very small doses required.

#### H. SUMMARY.

1. By means of a modification of Winogradsky's staining technique the changes in morphology of *Bacillus radicicola* in soil were followed. A regular cycle of changes was found, unbanded rods, cocci, and banded rods successively predominating in the soil. Increase in the percentage of cocci was associated with increased bacterial numbers and with the appearance of motile forms (Section C).

2. By modifying the liquid used to suspend the inoculum added to the soil, the time of appearance of cocci in predominance could be altered. In particular, inoculation of the soil with a bacterial suspension in milk + 0.1 per cent.  $\text{CaH}_4(\text{PO}_4)_2 + 2\text{H}_2\text{O}$  hastened the predominance of cocci and increased the percentage to which they attained (Section D).

3. When the centre of a petri dish of soil and sand is inoculated with a suspension of the bacteria, the latter commence, after a lag period, to spread radially at an approximate rate of one inch in 24 hours. The length of this lag period is apparently related to the time taken for cocci to predominate in the soil and is similarly effected by the nature of the inoculating fluid. The bacteria multiply rapidly in the soil into which they have recently spread, so that the nature of the inoculating fluid also exerts a remote influence on bacterial numbers. Thus inoculation of the soil with a bacterial suspension in milk containing 0.1 per cent.  $\text{CaH}_4(\text{PO}_4)_2 + 2\text{H}_2\text{O}$  results in a greater spreading of the bacteria through the soil and in greater multiplication at a distance from the point of inoculation than in the case when soil is inoculated with a suspension in milk alone (Section E).

4. Lucerne plants grown from seed inoculated with a suspension of bacteria in milk + 0.1 per cent.  $\text{CaH}_4(\text{PO}_4)_2 + 2\text{H}_2\text{O}$  showed a considerable increase in nodule numbers and in yield compared with plants from seed inoculated with a suspension in milk alone (Section F).

5. This effect was confined to the deeper portions of the root and therefore increased as the plants became older and roots developed in the deeper soil. This suggests that the additional nodule formation is due to the known effect of the phosphate in increasing the spreading of the bacteria (Section G).

REFERENCES.

- (1) Ball, O. M., 'Centralbl. f. Bakt.,' Abt. II, vol. 23, p. 47 (1909).
- (2) Beijerinck, M. W., 'Botan. Zeit.,' vol. 46, pp. 726, 758, 782, 798 (1888).
- (3) Bewley, W. F., and Hutchinson, H. B., 'Journ. Agric. Sci.,' vol. 10, p. 144 (1920).
- (4) Brunchorst, J., 'Berichte Deut. Botan. Gesell.,' vol. 3, p. 241 (1885).
- (5) Buchanan, R. E., 'Centralbl. f. Bakt.,' Abt. II, vol. 23, p. 59 (1909).
- (6) Frazier, W. C., and Fred, E. B., 'Soil Sci.,' vol. 14, p. 29 (1922).
- (7) Gangulee, N., 'Annals of Applied Biol.' (*in the press*).
- (8) Greig-Smith, R., 'Proc. Linn. Soc. N.S. Wales,' vol. 24, p. 653 (1900).
- (9) Kellerman, K. F., and Faucett, E. H., 'Science, N.S.,' vol. 25, p. 806.
- (10) Löhnis, F., 'Mem. Nat. Acad. Sci.,' Washington, vol. 16 (1921).
- (11) Löhnis, F., and Smith, N. R., 'Journ. Agric. Res.,' vol. 6, p. 675 (1916).
- (12) Morck, D., 'Diss. Phil.,' Leipzig, 44 pp. (1891).
- (13) Nobbe, F., 'Botan. Centralbl.,' vol. 68, p. 171 (1896).
- (14) Prazmowski, A., 'Landw. Vers. Stat.,' vol. 37, p. 161 (1890).
- (15) Wallin, I. E., 'Journ. Bact.,' vol. 7, p. 471 (1922).
- (16) Winogradsky, M. S., 'Chimie et Industrie,' vol. 2, No. 2 (1924).



*The Rôle of the Young Lucerne Plant in Determining the  
Infection of the Root by the Nodule-forming Bacteria.*

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Department).

(Communicated by Sir John Russell, F.R.S.--Received February 11, 1929.)

[PLATE 31.]

Observations have been made at Rothamsted over a period of about five years upon the development of nodules on young seedlings of lucerne (*Medicago sativa*, L.). Some thousands of seedlings have been examined in various experiments and it was found to be the rule that the first appearance of nodules coincided with that of the expansion of the first true leaf. When lucerne is sown under summer glasshouse conditions, in pots of soil or sand, the seedlings are up in from 3 to 5 days, and in 6 to 8 days the first true leaf becomes visible. This is at first closed, but in 8 to 12 days from the date of sowing it opens out (fig. 1).

The following experiment illustrates the relationship between the opening

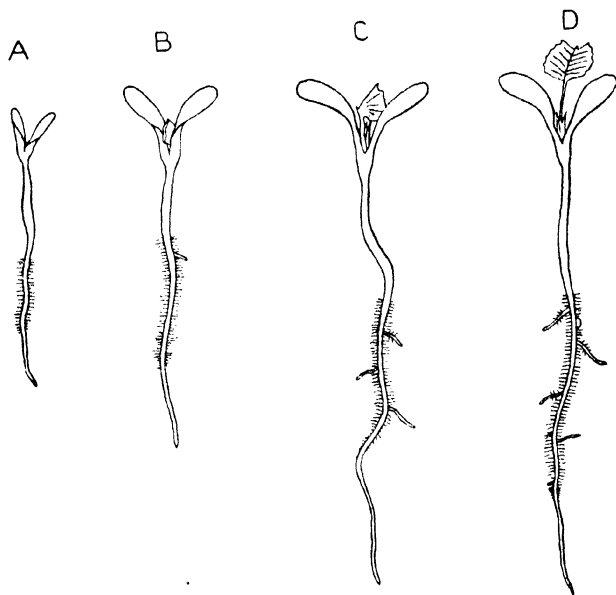


FIG. 1.

of the first leaf and the appearance of nodules. Lucerne seed, inoculated with nodule bacteria was sown in 12 pots, each containing about 8 pounds of sand, and these were watered with a plant-culture solution free from nitrogen.\* Five days after sowing the seedlings were up, and in another 4 the first true leaf, still closed, could be seen on most of them. The appearance of nodules and the opening of the first true leaves is shown in fig. 2, where each point represents observations made upon 20 seedlings, 10 from each of duplicate pots. The general agreement in the time of appearance of nodules and in the opening of the first true leaves is evident.

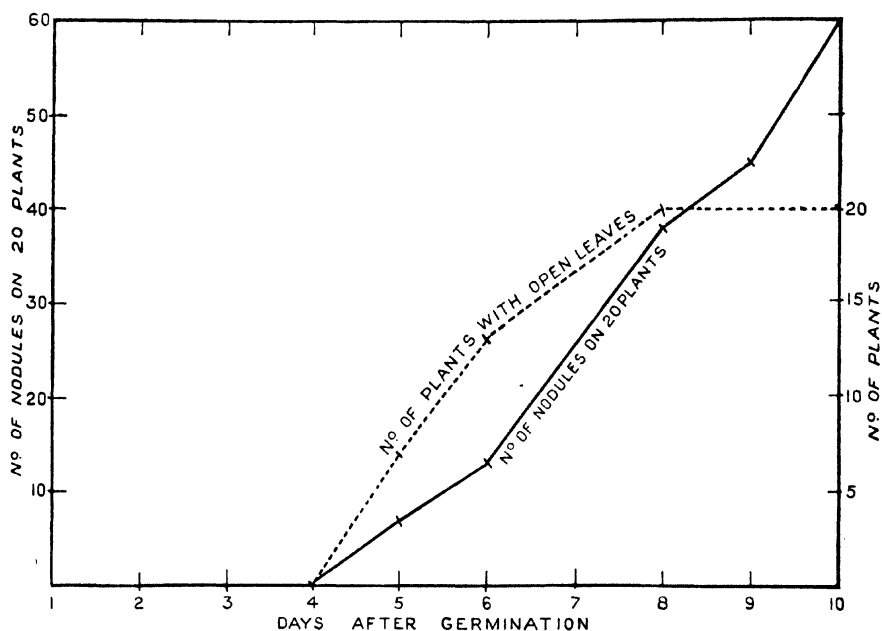


FIG. 2.

The closeness of this agreement can be more clearly appreciated when, instead of making such counts from samples of a seedling population, the course of development and nodule appearance is followed on the same individual plants grown in water culture. For this purpose seedlings were grown in test-tubes containing the food solution referred to above. Observations were made at intervals on the same 20 seedlings. The appearance of nodules is

\* The plant-culture solution used throughout this work had the following composition :—  
 Water, 1000 c.c. ; KCl, 0.74 grs. ;  $K_2HPO_4$ , 0.3 grs. ;  $KH_2PO_4$ , 0.3 grs. ;  $MgSO_4 \cdot 7H_2O$ , 0.5 grs. ; NaCl, 0.5 grs. ;  $CaSO_4$ , 0.5 grs. ;  $FeCl_3$ , 0.04 grs.

shown in fig. 3, where the age of each seedling is taken from the date of the opening of the first true leaf. Only one nodule had appeared on the day before this opening, although the roots had been in contact with a suspension of the bacteria for 9 days. Four more nodules appeared on plants the same day that the leaf opened and 8 more the following day.

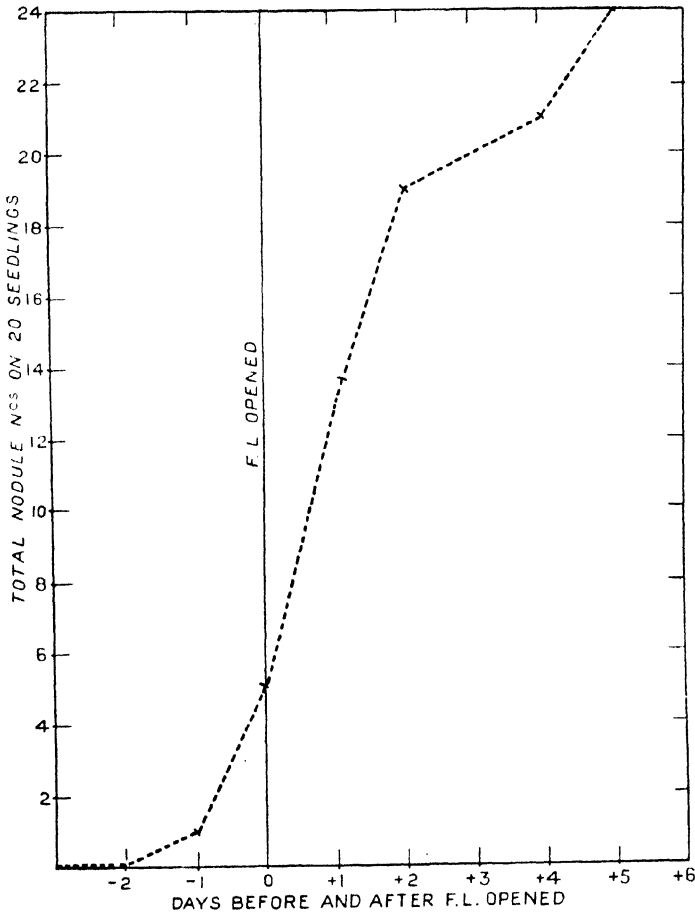


FIG. 3.

The appearance of nodules in another similar set of water cultures is shown in fig. 4, where the appearance of lateral roots and of the first leaf still in a closed condition are also recorded. In view of the comparisons sometimes drawn between nodules and lateral roots—it is interesting to note that the time of first appearance of the two do not coincide. The experiment also shows

that appearance of the first nodules corresponds with the *opening* of the first leaf and not with its emergence from the growing point.

In the last set of cultures the roots had been in contact with a suspension of nodule bacteria for 10 days before the opening of the first leaves and the

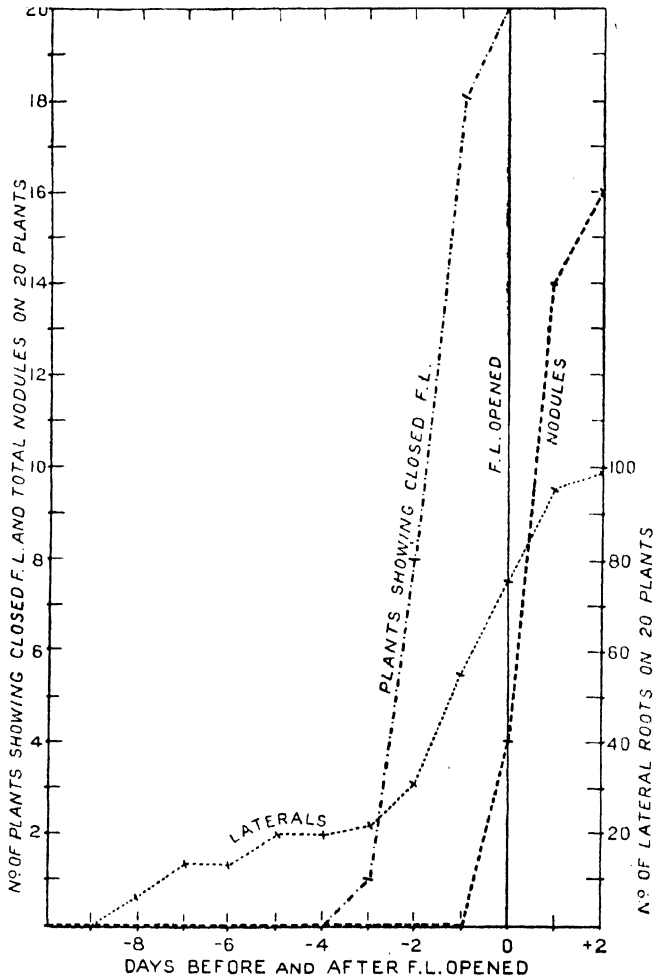


FIG. 4.

appearance of nodules. In seeking an explanation of this lag it seemed necessary to discover first whether the organisms fail to enter the root hairs during the "cotyledon stage" or whether, having entered the plant, they are unable to induce the formation of a nodule. Owing to the transparency of the ro

cortex, it is possible to see a nodule in the very early stages of its growth. When a root hair has been infected the infection strand passes through the cortical cells as far as the endodermis and, along its course, the cortical cells become more densely protoplasmic and commence to divide (fig. 5). In such a very young stage the cells affected are only about 25 in number but their increased opacity renders the "nodule" clearly visible in the living root. The absence of visible nodules thus implies that this earliest stage has not even been reached.

It was therefore a question whether the organism enters the root during the

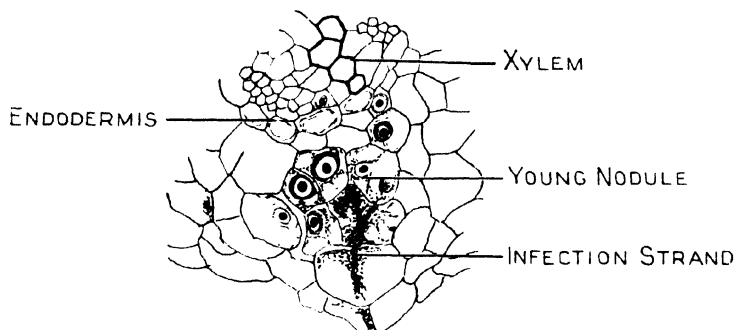


FIG. 5.

cotyledon stage or whether it is then normally unable to infect the root hairs. To determine this, seedlings were grown aseptically in boiling tubes containing a plant-food solution in agar. Twelve seeds, the coats of which had been sterilised by immersion in absolute alcohol followed by 0.2 per cent.  $\text{HgCl}_2$  washed off with sterile water, were sown in each of 4 tubes. When they had germinated, a suspension of the nodule organism was introduced and the roots were examined at daily intervals, a search being made for infected root hairs. Within 4 days of germination most of the plants showed the first leaf in a closed state. Four days later the majority had the first leaf open. During the 8 days that thus elapsed between inoculation and the opening of the first leaf the roots were surrounded by large numbers of organisms, many in a motile state, but not a single root hair containing an infection thread was seen. The absence of the bacteria other than the nodule organism was shown by plating at the end of the experiment. One day after the opening of the first leaf on the majority of plants about 2 per cent. of the root hairs contained infection threads, which were easily seen owing to their high refractive index.\*

\* Previous observations on slightly more advanced seedlings showed 4 per cent. of the hairs with infection threads (Thornton (1)). Pierce (2) however, claims to have obtained a much heavier infection on roots placed between layers of filter paper.

The evidence thus indicates that the bacteria do not normally enter the root before the first leaf opens.

This delay in the infection of the root until the opening of the first leaf may be wholly or partially due to some factor in the plant's physiology or may represent a period of development of the nodule organisms outside the plant necessary to enable them to reach an infective condition (*cf.* Thornton and Gangulee (3)). If the delay were solely due to the organisms, the time taken for nodules to appear should be independent of the age of the seedlings. The following experiment was therefore made to determine how soon after their addition to the sand the nodule bacteria can produce nodules on older, as well as on the young seedlings.

Lucerne seed was sown in 10 pots of sand sterilised with steam and watered with sterile plant-food solution. The seed coats were sterilised as described above and 2 pots (Series A) were left uninoculated to serve as controls against contamination. Four pots (Series B) were sown with uninoculated seed and, after 3 weeks' growth, when the seedlings had a widely expanded first leaf, these pots were inoculated by pouring on a thick suspension from a young culture of the nodule organism. Twenty seedlings from this series were examined 3, 5, 7 and 9 days after inoculation. The mean number of nodules per plant on each occasion is shown in fig. 6. A considerable number of nodules had appeared on the fifth day after inoculation.

That these nodules were produced by the culture added was shown by the almost complete absence of nodules on the control plants, examined at the end of the trial. On the day on which Series B was inoculated another 4 pots (Series C) were sown with seed and similarly inoculated. Seven days later the first leaves of the series were showing but had not opened. On the seventh, ninth, eleventh and thirteenth days after sowing and inoculation 20 seedlings from these pots were examined. The course of nodule appearance is shown in fig. 6. The appearance of the nodules was delayed until the true leaves opened after 9 days although Series B showed that the culture was capable of producing nodules within 5 days from the date of inoculation. The delayed appearance of nodules must therefore be due to the plants.

The rule that no nodules appear before the expansion of the true leaves is not absolute. When a large number of seedlings are grown together in sand culture there is a period during which the first leaf has opened on some plants but not on others. At this time nodules appear on the former plants but also on a few of the plants whose first leaves have not yet opened. Thus in the pot experiment illustrated in fig. 2 the examination made on the fifth day

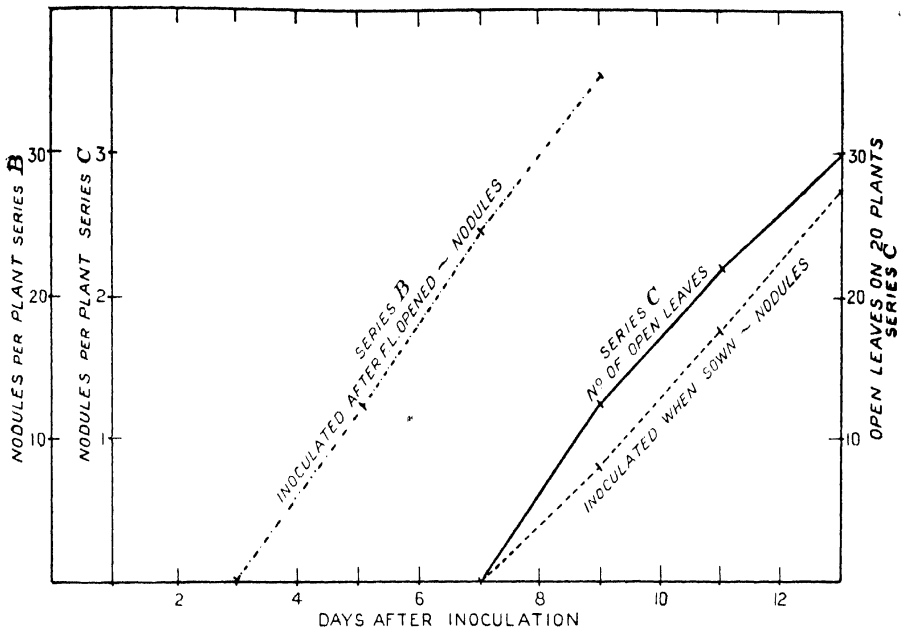


FIG. 6.

after germination showed the following distribution of nodule-bearing plants :—

Nodule-bearing plants, with leaf open .....	5
"    "    without open leaf .....	2
Plants without nodules, with leaf open .....	3
"    "    without open leaf .....	10

Other examples are shown in the control series shown in Tables I and II. In normal cases only a small proportion of such younger plants bear nodules. In one pot experiment, however, a wholly abnormal percentage of nodules appeared on plants whose first leaf had not yet opened. In this experiment the inoculated seed had been sown thickly in sand watered with plant-food solution. The seed germinated unevenly, some plants showing open leaves after 9 days, while 12 days after sowing only about half the plants had the first leaf open. On the twelfth day 20 seedlings with open leaves and 20 with leaves still closed were taken at random from 4 pots and examined. The former had a total of 96 nodules while the latter had 64.

This experiment was abnormal in that, owing to uneven germination, young plants with the first leaf unopen grew for some days mingled with older plants

in which the leaf had expanded. An experiment was therefore made in which this condition was deliberately reproduced. Four pots of sterilised sand, watered with sterile plant-food solution, were prepared, and in two of these lucerne seed sterilised as above described was sown thinly. Fourteen days later the seedlings bore large expanded first leaves. Fresh seed was then sown in these pots among the seedlings and at the same time the two remaining pots were sown and all four pots inoculated with a suspension of the nodule bacteria. After 10 days the later sown seedlings showed the first leaf still closed. Fifty of these younger seedlings from each pot were examined with the result shown in Table I.

Table I.—Effect of the Presence of Older Seedlings upon the appearance of Nodules on Young Seedlings.

	Number of nodules on 50 seedlings with the first leaf closed.		
	Plot 1.	Plot 2.	Mean.
Only young seedlings present .....	6	5	5.5
Young seedlings growing amongst older.....	38	35	36.5

The presence of the older plants had induced the formation of a considerable number of nodules on the young plants growing amongst them at the stage when only occasional nodules would normally have appeared. This influence of the older plants upon their surroundings suggests the extrusion of some substance from the roots. To investigate this possibility an attempt was made to extract the solution from sand cultures in which lucerne seedlings had been growing. Pyrex glass troughs, 10 × 5 inches in area and 2½ inches deep, were filled with sand watered with equal volumes of plant-food solution and sown thickly with lucerne seed. When the seedlings were in the cotyledon stage the contents of one trough was carefully packed into a glass tube, 2 inches in diameter and 3 feet 6 inches long, clamped in a vertical position and fitted at the lower extremity with a perforated rubber bung and a glass delivery tube. The solution was extracted with the pressure of a head of distilled water, about 1 litre of the solution being thus obtained (Extract A). When the seedlings in the second trough had well-expanded first leaves the solution from this culture was extracted in a similar manner (Extract B). The two extracts were sterilised in the autoclave. Their action was tested in the following manner.

Six pots were filled with sand, sown with lucerne seed and watered with plant-food solution. When the seedlings were up 2 pots were inoculated with a suspension of the nodule bacteria in plant-food solution made up in distilled water, 2 pots were inoculated with a suspension in plant-food solution made up in Extract A and 2 pots with a suspension in a similar solution made with Extract B. 15° c.c. of each solution were thus added per pot. Ten days after sowing some of the seedlings showed first leaves just open. Twenty plants with the leaves open and 20 with them still closed were examined from each pot, with the results shown in Table II.

Table II.—Effect of the Solution Surrounding the Roots.

	Number of nodules upon 20 plants.					
	Plants with first leaf open.			Plants with first leaf closed.		
	Pot 1.	Pot 2.	Mean.	Pot 1.	Pot 2.	Mean.
Watered with food solution alone	25	34	29.5	0	5	2.5
Watered with food solution + Extract A	20	20	20.0	0	7	3.5
Watered with food solution + Extract B	32	34	33.0	18	13	15.5

The extracts have produced no effect on the nodule numbers on such plants as had the leaves already open. The Extract A produced no significant effect at all, but Extract B, prepared from sand in which older seedlings were growing, caused a marked development of nodules on seedlings whose first leaf had not yet opened, increasing their numbers six-fold as compared with the control plants in the same stage. It would thus appear that, when the lucerne seedlings have attained a stage of development marked by the opening of the first true leaf, some substance is extruded from the roots which stimulates the infection of the root hairs by the nodule bacteria.

In order to observe whether the extracts had any influence upon the growth of the nodule organism, the plant-food solution plus 1.5 per cent. agar was made up with distilled water, with Extract A and with Extract B. A similar set of media were made up containing 1 per cent. saccharose. The media were sterilised in the autoclave, poured into sterile petri dishes and streaked with a 2-day old culture of the lucerne nodule organism. After one week's incubation

at 25° C. scarcely any growth appeared on the media without saccharose. In the presence of saccharose no growth showed on the medium made up with distilled water, a slight opaque growth on the medium made up with Extract A and a considerable slimy growth on the medium containing Extract B. (See Plate 31.)

The substance formed by the older seedlings thus has an effect upon the growth of the nodule bacteria, but this effect would seem not to consist in supplying energy material since it showed itself only when sugar was added. The extracts did not reduce Fehling's solution and tests for sucrose and for pentoses were also negative. A pot experiment was made in order to see whether the presence of an amino acid would induce nodule formation on seedlings before the first leaves opened. Inoculated seedlings were given food solution alone and food solution containing 0·01 per cent. and 1·0 per cent. asparagine. In neither case were the nodule numbers increased by asparagine.

The manner in which the extract stimulates infection is not at present clear. Its effect in increasing the number of bacteria may be of some importance but observations of root hairs in plants grown on agar, referred to above, tend to show that this cannot be a limiting factor since seedlings in the cotyledon stage do not show infected root hairs even in the presence of considerable numbers of the bacteria. It is observable that in this case the bacteria exist either as individuals or else in loose clumps among the root hairs, whereas the infection of a root hair is preceded by the formation of a minute colony of bacteria imbedded in slime, showing as a bright refractile spot on the wall of the root hair. Such spots are illustrated by Marshall Ward (4). The fact that a slimy growth of the bacteria was induced when these were growing on agar containing Extract B may therefore be relevant.

The coincidence in time of nodule appearance with the opening of the first leaf raises the question as to whether these two events are causally connected or whether they are both the effect of some other physiological change in the seedling. To answer this question the course of nodule development was followed on seedlings from which the true leaves were removed. Lucerne seedlings were grown in test-tubes containing plant-food solution inoculated with a suspension of the nodule bacteria. In one series the true leaves were cut off as soon as they appeared; in the second series the cotyledons were cut off but the true leaves allowed to develop, and in the third, control series, the true leaves and cotyledons were left. The course of nodule development is shown in fig. 7.

On the control plants the appearance of the nodules followed the opening

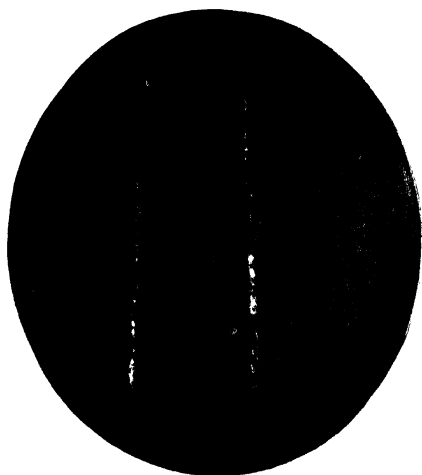


FIG. 1.—Lucerne Nodule Bacteria, growing on Agar with Nutrient Salts and Saccharose.

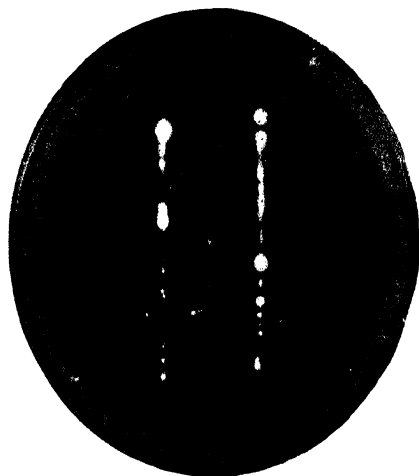


FIG. 2.—Lucerne Nodule Bacteria, growing on Agar with Nutrient Salts, Saccharose and Extract A.

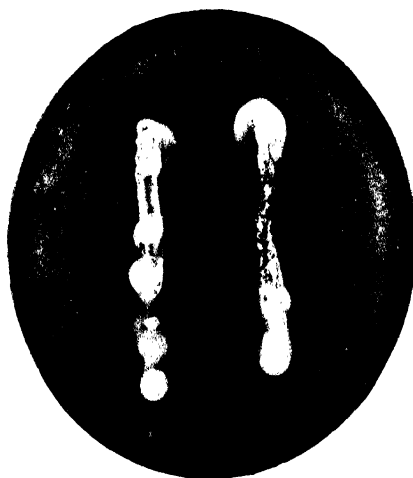


FIG. 3.—Lucerne Nodule Bacteria, growing on Agar with Nutrient Salts, Saccharose and Extract B.



of the true leaves. In the series with the cotyledons cut, the opening of the true leaves followed a course similar to that shown for the control plants, but

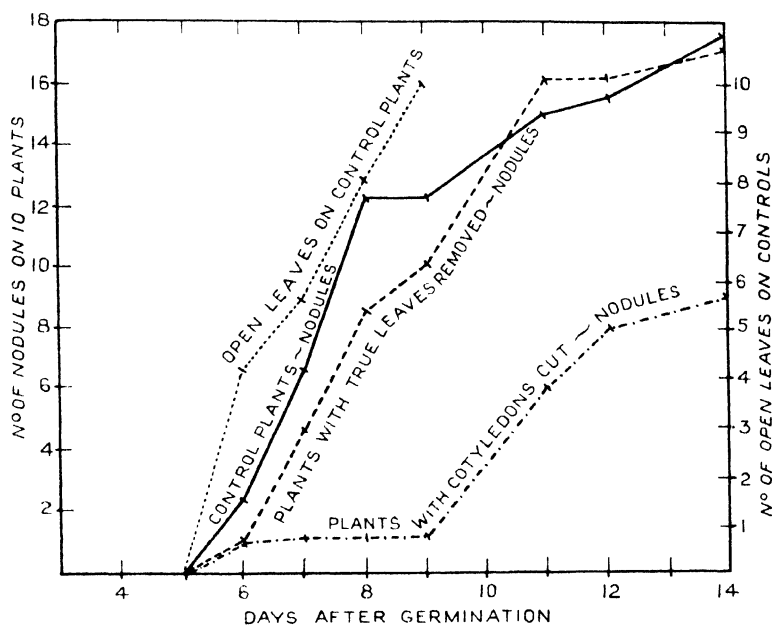


FIG. 7.

the development of nodules was delayed until considerable growth of the true leaves had occurred. This indicates that the passage into the root of substances from the cotyledons is one of the factors controlling the early appearance of nodules. Where the true leaves were removed, however, the normal course of nodule appearance was not significantly altered.

The substance affecting nodule formation is therefore not produced in the true leaf at the time of its expansion since its action is unaffected by the removal of the leaf. It seemed possible that it is formed in the growing point of the top. In the hope of testing this possibility, a sand culture experiment was made, in which seedlings were grown in three series of duplicate pots, those in the first series having the leaves cut before expansion, those in the second series having the terminal bud cut out and those in the third control series being untouched. Three weeks after sowing the control plants had one expanded leaf and, in the majority, a second closed leaf just appearing. Fifteen plants from one pot of each series were then washed and their nodules counted. Twenty days later a second similar examination was made. There were no significant

differences in nodule numbers between any of the series. The removal of the terminal bud therefore did not affect the number of nodules formed. It was found, however, that in most of the plants two fresh buds were regenerated at the bases of the cotyledons, so that this series merely showed that severe damage to the shoot meristem does not affect the formation of nodules. The results from the first series, on the other hand, confirm the previous experiment in showing that the appearance of nodules is not checked by removal of the first leaf before it opens. It would seem therefore that the opening of this leaf coincides with some other change in the physiology of the seedling, resulting in the extrusion of the substance which stimulates infection by the nodule organism.

*Summary and Abstract.*

1. The appearance of nodules on seedlings of lucerne (*Medicago sativa*, L.) coincides with the opening of the first true leaf.
2. There is evidence that before this leaf opens the nodule bacteria do not as a rule infect the root hairs.
3. The delayed infection is due to the plant and not to any delay in the development of infective power by the bacteria.
4. When young inoculated seedlings, whose first leaves are still closed, are grown intermingled with older plants, a considerable number of nodules will develop on them, although scarcely any are formed on control seedlings of the same age, grown by themselves.
5. The solution surrounding the roots of seedlings whose first leaves are expanded has an influence in stimulating the appearance of nodules on younger seedlings, and increases the growth of the nodule organism on agar. The solution surrounding the roots of younger seedlings has no such effect.
6. The active substance inducing nodule appearance when the first leaf opens is not formed in this leaf, since the removal of the leaves while still closed has no effect on nodule appearance.

REFERENCES.

- (1) Thornton, H. G., 'J. Agric. Sci.,' vol. 19 (1929), *in the press*.
- (2) Pierce, G. J., 'Proc. Calif. Acad. Sci.,' Ser. 3, vol. 2, p. 295 (1900).
- (3) Thornton, H. G., and Gangulee, N., 'Roy. Soc. Proc.,' B, vol. 99, p. 427 (1926).
- (4) Marshall Ward, H., 'Roy. Soc. Proc.,' B, vol. 46, p. 431 (1890).

*The Influence of the Host Plant in Inducing Parasitism in Lucerne  
and Clover Nodules.*

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[PLATES 7-11.]

*A.—Introduction.*

The relationship of the nodule organism to its host plant has been much discussed, some authors regarding it as an instance of true symbiosis, while others regard the organism as a parasite to which the host plant offers a certain resistance. Failures to obtain inoculation of legumes with strains of nodule bacteria belonging to a different inoculation group may be regarded as examples of such resistance. Even where nodules are formed, the fixation of nitrogen and the benefit derived by the host plant varies according to the strain of the nodule organism concerned (Stevens (1) and Wright (2)). Some strains, while producing nodules, cause no increase in growth or nitrogen content in the host plant (3) and (4). It is uncertain whether the resistance of the host plant prevents the normal functioning of such strains, or whether they are actively parasitic on the nodule tissue.

Strains of the nodule organism thus differ in their relationship to the host plant. The behaviour of a single strain in the tissues may also be altered by the condition of the host plant. Thus, when *Vicia faba* is grown in a boron-deficient solution, the conducting tissue develops abnormally, so that the vascular supply to the nodules is either absent or incomplete. In such nodules the bacteria fix but little nitrogen and destroy the host cells in which they lie, although the same strain in healthy plants behaves normally and fixes appreciable amounts of nitrogen (Brenchley and Thornton (5)). It is thus possible experimentally to alter the relationship between the host plant and the bacteria, so that a strain of the latter which is normally beneficial to its host becomes actively parasitic. It was suggested that, in normal nodules, the bacteria derive their energy material from the carbohydrates conveyed to them along the vessels, but that in boron-deficient plants they are to a large extent deprived of their carbohydrates, owing to failure of the vascular supply, and derive energy by attacking the host protoplasm. If this hypothesis be correct, it

should be possible to induce the change from symbiosis to parasitism by cutting off the carbohydrate supply in other ways, for example by keeping the plants in darkness.

*B.—Infection of Darkened Seedlings.*

The following experiment was made to ascertain whether nodules could be formed on plants kept in the dark. Eighteen wide test-tubes were half filled with a mixture of equal parts of garden soil and sand and sterilised in the autoclave. Each was inoculated with a suspension of a 3-day old culture of the lucerne nodule organism. Twenty seeds of lucerne (*Medicago sativa*, L.) the coats of which had been sterilised by immersion in absolute alcohol followed by 0.2 per cent.  $\text{HgCl}_2$ , washed off with sterile water, were placed in each tube. Six tubes were kept in the light, six in darkness, and the remaining six in light until the seedlings showed the first true leaves open, after which they were kept dark. Germination took place in 7 days and, in the lighted seedlings, the first true leaves opened 5 days later. Three weeks after sowing 20 plants from each of duplicate tubes were examined. The development of nodules is shown in Table I.

Table I.—Effect of Light and Darkness on Nodule Formation.

	Nodules on 20 plants.			Mean length of nodules in mm.	Mean breadth of nodules in mm.
	Tube 1.	Tube 2.	Mean.		
Plant kept dark .....	3	4	3.5	0.25	0.25
Plants kept dark after true leaves opened .....	17	15	16	0.4	0.5
Plants kept in the light .....	42	51	46.5	1.0	0.75

Complete darkening of the plants almost entirely stopped the appearance of nodules. Darkening after the opening of the first true leaf greatly reduced nodule formation. Some of the nodules in this series probably appeared while the plants were still exposed to light, for although very few nodules develop before the true leaf opens, this rule is not absolute (Thornton (6) ).

The following experiment was made in order to ascertain whether nodules continue to be formed after plants are placed in darkness. Sixteen wide test-tubes containing an agar medium, made up with plant nutrient solution.\*

\* The medium had the following composition :— $\text{K}_2\text{HPO}_4$ , 0.5 grs. ;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.2 grs. ;  $\text{NaCl}$ , 0.1 gr. ;  $\text{Ca}_3(\text{PO}_4)_2$ , 2.0 grs. ;  $\text{FePO}_4$ , 1.0 grs. ;  $\text{FeCl}_3$ , 0.01 gr. ; agar, 10 grs. ; water, 1000 c.c.

were sterilised and each was sown with two lucerne seeds, the coats of which had been sterilised as described above. The seeds germinated in 3 days and the tubes were then inoculated with a 10-day old culture of the lucerne nodule organism. Two weeks later most plants had the second true leaf open and nodules had begun to form. 18, 19 and 28 days after germination, pairs of duplicate tubes were removed and placed in the dark. The number of nodules that appeared in these tubes before and after the tubes were darkened is shown in fig. 1. Darkening did not immediately stop nodule formation, one or

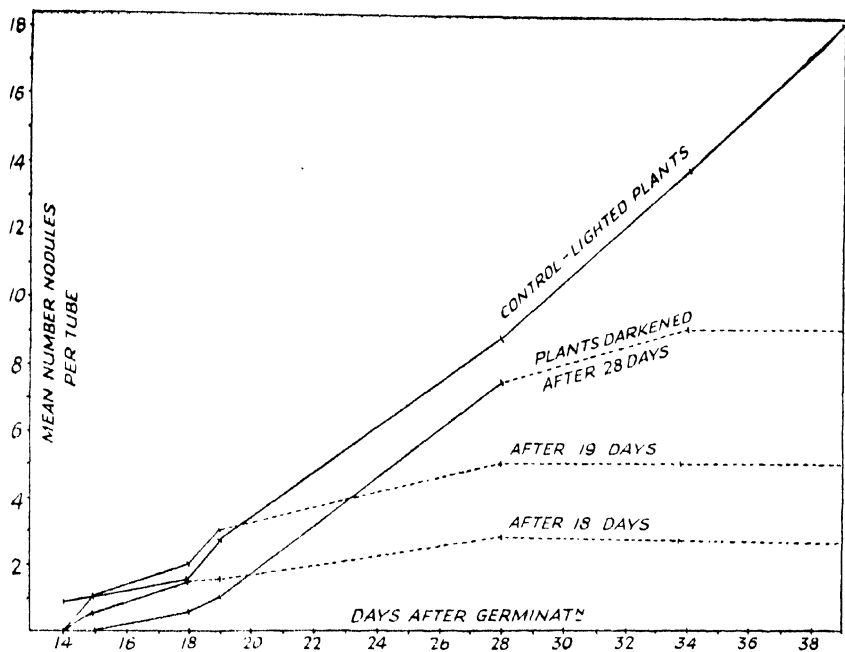


FIG. 1.—Appearance of Nodules on Seedlings grown in Light and in Darkness.

two nodules appearing after this was begun, but soon the process was completely stopped.

The arrest of nodule formation is presumably due to the absence of some factor which in lighted plants assists the development of nodules, since observations described below show that the protoplasm and cell walls of the host are not made more resistant to the nodule bacteria by darkening the plant. The plants apparently contained a small reserve of this factor at the time of darkening, no further nodules being formed when this was used up. The factor must be present in small quantities in the seed, since a few nodules

developed on plants grown entirely in the dark (Table I). When inoculated seedlings grown in the light are deprived of their cotyledons, the appearance of nodules is delayed until the true leaves are well developed (Thornton, 6). A substance essential to nodule formation is thus present in the cotyledons and is presumably also formed in the true leaves. It is perhaps the lack of this substance in darkened seedlings that stops the formation of nodules. The fact that cell division ceases in the roots of such seedlings suggests that the substance acts by furthering this process.

*C.--The Degeneration of Nodules on the Roots of Darkened Seedlings.*

The nodules on the roots of plants placed in the dark soon ceased to grow and remained small (Table I). The plants placed in darkness in the second experiment bore some nodules which had appeared when the plants were lighted. These nodules, and others formed after the plants were darkened, were fixed in Bouin's fixative (7) and serial sections  $5\ \mu$  thick were cut and stained with iron hæmatoxylin and orange G. These preparations were compared with others from a control series of nodules taken from plants kept all the time in the light.

The development of the nodule on normal lucerne seedlings grown in light is as follows. The bacteria infect a root hair, forming within it one or more "infection threads" containing the organisms in the short uniformly-staining rod stage. Infection threads penetrate cells as far as the inner layers of the cortex but do not penetrate the endodermis. Along the course of the threads the cells that have been penetrated and those adjacent to them become more densely protoplasmic, their nuclei increase greatly in size, and active cell division commences. The cell division thus begun produces a mass of meristematic tissue, characterised by protoplasmic cells with large nuclei (Plate 7, fig. 1). Through these the infection threads ramify.

Infected cells are sometimes seen in a state of division and in such cases the mechanism of the mitotic spindle does not appear to be damaged by the presence of the infection thread of bacterial zoogloea within the cell. The thread often grows towards and applies itself to the host cell nucleus, which, however, remains apparently undamaged by this.

When the nodule reaches a diameter of about 1 mm., the cells in the proximal region cease to divide (Plate 7, fig. 2). At the distal end, however, cell division continues, a meristem cap being thus formed, which causes the nodule to increase in length and to become roughly cylindrical. At about this time vascular strands grow out into the nodule cortex in the manner described in

detail in the case of *Vicia* by Brenchley and Thornton (5). Swelling of the infected cells and a rapid multiplication of the bacteria within them accompanies the growth of the vascular strands. Save in old nodule tissue the bacteria do not normally appear to injure the cells in which they lie, the nucleus of the host cell, and even the cytoplasmic strands running from it, being undamaged by them (Plate 8, fig. 1). Degeneration of the cell contents occurs only in old infected cells. The bacteria that lie in the cytoplasm show a progressive change in appearance, increasing in size and becoming banded (Plate 9, fig. 1). During the growth of the nodule the young cells in the inner layers of the meristem cap become infected by ramification of the infection threads, portions of which also persist in the proximal regions of the nodule and eventually play a part in its decay.

When lucerne seedlings are grown entirely in the dark such nodules as appear do not attain a diameter greater than 0.5 mm., and remain in the roughly spherical stage reached in normal nodules about the first or second day. Sections show that such nodules, when about 2 weeks old, consist of small cell tissue, the cells of which have ceased to divide (Plate 7, fig. 3). The cytoplasm has shrunk to a thin layer lining the cell walls and the nuclei are very small. These changes are not due to the action of the infecting bacteria, since they occur in other meristem tissue, such as young lateral roots. The infection threads are more numerous than in normal nodules. They show a greater tendency to swell out into zooglœal masses. They tend as usual to grow up to and apply themselves against the nucleus of the host cell; the latter however does not remain undamaged, but in many cells becomes surrounded by a mass of bacterial zooglœa and progressively degenerates, first losing its shape and structure and finally disappearing. This destruction of the nucleus is not seen in uninfected meristem tissue and is thus due to the presence of the bacteria, which have become actively parasitic (Plate 7, fig. 4).

On seedlings kept at first in light and then in the dark, nodules that had begun to develop in the light continued to increase in size for a few days and then stopped growing, cell division being arrested. In the younger portions of such nodules the infection threads tend to swell out into zooglœal masses and to attack the nuclei of the host cells, as was noted above. In the older swollen cell tissues the cytoplasm is destroyed, the nucleus shrivels up and vanishes, and the bacteria collect in dense masses in the cells. (Compare figs. 1 and 2, Plate 8.) The bacteria in such tissues change from swollen banded rods to much smaller rods and coccoid forms.

In nodules from darkened seedlings the bacteria do not remain confined to

the cells, but invade the intercellular spaces and the middle lamellæ of the cell walls. In young healthy nodules such invasion does not occur, the bacteria being entirely intracellular, except at the point where the infection threads cross the cell walls. In nodules from darkened seedlings, the points at which infection threads cross the cell wall become centres from which bacteria invade the intercellular spaces (Plate 8, fig. 4) and, by attacking the middle lamellæ of the walls (Plate 8, fig. 3), gradually cause the nodule tissue to disintegrate.

#### D.—*Decay of Old Nodules.*

The observation that in these abnormal nodules the bacteria tend to attack the middle lamella of the cell wall, has a special bearing upon the problem of the decay of old nodules on normal plants. In perennial legumes, such as lucerne, most of the nodules decay in the autumn, a fresh batch forming on young roots the following year. This decay takes place most rapidly in the interior of the nodule, so that decaying nodules are often hollow inside while the cortex is intact. Milovidov (8), in his observations on the histology of clover nodules, describes the older proximal region of the nodule as having the intercellular spaces invaded by coccoid rod forms, which eventually cause disintegration of the tissue. In lucerne, as in clover, coccoid rods invade the intercellular spaces and the middle lamellæ of the cell walls, in older parts of the nodules, towards the end of the season.

It was necessary to make certain that these bacteria were in fact the nodule organism, and were not contaminating organisms which had entered the nodule from outside. To decide this point, lucerne seedlings were grown in wide test-tubes containing the agar medium described above, which were sterilised in the autoclave and sown with seeds whose coats were also sterilised. When the seeds had germinated, 15 of the tubes were inoculated with a culture of the lucerne nodule organisms and 5 were left uninoculated, to serve as controls. These latter remained free from bacterial growth. The seeds were sown in April and, in the inoculated tubes, plentiful nodule development occurred. Towards the end of the summer the plants began to lose their leaves, and in September platings from nodules out of five of the tubes were made on lucerne-root extract agar and on nutrient agar. In no case did colonies other than those typical of the nodule organisms appear. A number of nodules from these same tubes were fixed in Bouin's fixative, paraffin sections were made and stained with iron hæmatoxylin and orange G.

In these old nodules the centre was usually hollow, the space being surrounded by collapsed and disintegrating tissue (Plate 9, fig. 4). The stages of this

disintegration could be followed by studying regions of different ages in the nodules. In the earliest stage, coccoid rods invade the intercellular spaces and the middle lamella of the cell wall. The invasion commences at the point where an infection thread crosses a cell wall. There are frequently pieces of infection thread persisting in the swollen cells containing bacteroids, and, in these threads, the bacteria remain in the form of coccoid rods. The bacteria that invade the middle lamella and the intercellular spaces multiply and remain in this form (Plate 9, fig. 2). The cell walls invaded by the bacteria swell out and eventually collapse (Plate 9, figs. 3 and 4).

Meanwhile progressive degeneration of the cell contents of the swollen cells and of their contained bacteroids takes place. The nuclei lose their shape and disintegrate and the cytoplasm shrinks to a small lump. The bacteria in the cytoplasm, which in younger tissue consist of banded rods (Plate 9, fig. 1) break up into darkly-staining granules (Plate 9, fig. 2) which eventually disappear or at any rate lose their staining properties.

In the final stages of decay, collapse and disintegration of the tissue results in the formation of a hollow space surrounded by fragments of cell walls and zoogloeal masses of bacteria in the coccoid rod stage (Plate 9, fig. 4). When the nodule breaks up, it is presumably these bacteria which escape into the soil to infect fresh roots. Continuance of the race is therefore brought about by the descendants of the originally small numbers of bacteria which have persisted within the infection threads and which escape and multiply in the intercellular spaces. It is not brought about by the bacteria that have multiplied in the cytoplasm and carried out the nitrogen fixation, since these degenerate and apparently come to nothing. The life-cycle of the bacteria within the lucerne nodule may thus be represented diagrammatically as in fig. 2.

The process of decay was also followed in old clover nodules. For this purpose plants were grown aseptically as above described. Some tubes were kept uninoculated as controls, and these showed no signs of bacterial growth. Other tubes were inoculated with a pure culture of the clover nodule organism, and plantings from the contents of these tubes, made at the conclusion of the experiment, showed only colonies typical of the nodule bacteria. Nodules were fixed and sections made and stained as described in the case of lucerne nodules.

In clover, as in lucerne, bacteria in the coccoid rod stage, derived from the old infection threads, invade the intercellular spaces and the middle lamellae of the cell walls, from the points at which infection threads enter the cell

(Plate 10, fig. 3). Milovidov (8) states that cyst-like swellings, formed on the infection thread in young cells, act as reservoirs from which coccoid rods

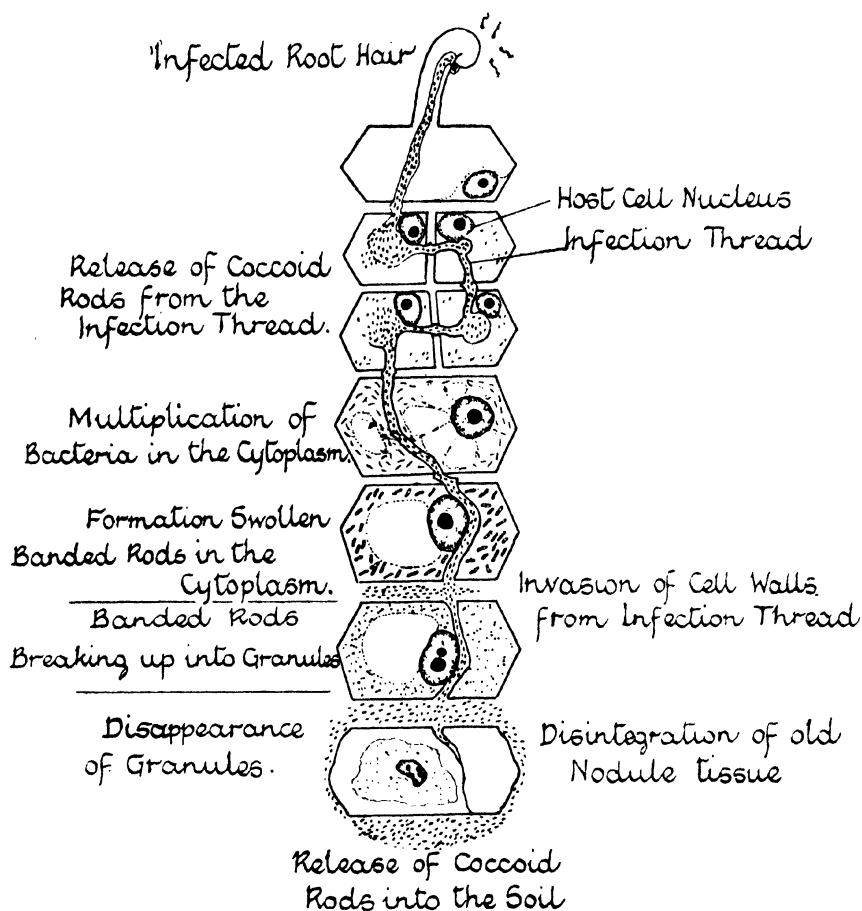


FIG. 2.—Life history of *Bacillus radicicola* in the tissues of the Lucerne Nodule.

invade the intercellular spaces in older tissue. The old infection threads themselves were centres from which the invasion took place in the material here studied. The infected cell walls swell up and the coccoid rods multiply therein (Plate 10, fig. 4). The bacteria in the cytoplasm, which become more swollen than in lucerne (Plate 10, fig. 1), break up into granules (Plate 10, fig. 2), which eventually lose their staining properties. The nuclei of the infected cells degenerate, losing their shape and structure and finally disappearing, and the cytoplasm shrinks to a lump in the centre of the cell.

*E.—Discussion.*

The behaviour of the nodule bacteria within the tissues is liable to be altered by the condition of the host plant and to change during the life of a single nodule. Three conditions are known under which a strain of nodule organism, known to exist normally within its host as a beneficial symbiont, will become actively parasitic and destroy the tissues of the same host. The first condition is induced by growing the plants in boron-deficient solution (Brenchley and Thornton (5)) so that the vascular supply to the nodule is absent or defective. The second condition is obtained by growing the host plant in the dark and thus preventing photosynthesis. In both conditions the normal carbohydrate supply to the nodule is cut off; in the first at the nodule itself and in the second in the leaves. It is natural to suppose that this shortage of carbohydrate is in both cases the cause of the similar change to parasitism, and that when no carbohydrate is brought to them, the bacteria obtain their energy by attacking the nucleus, cytoplasm, and cell walls of the host tissue.

The fact that the bacteria do not attack the host cells in normal young nodules is not, however, fully explained by this hypothesis. The host tissues provide a source of energy which they are able to utilise, since they do so when the carbohydrate supply is deficient. Why do not the bacteria in the healthy nodule increase up to the limit set by the total energy supply, consuming both the carbohydrates brought to them and also the host tissues? There is presumably some other factor which limits the bacterial population to a size which can obtain the needed energy from the carbohydrates normally supplied by the leaves. The air supply is a factor likely to limit the bacterial numbers in nodule tissue.

Observations showed that the functioning of nodules was closely dependent on good aeration. Seedlings were grown in wide test-tubes each containing a column of agar medium about  $2\frac{1}{2}$  inches deep. Under such conditions plants in some tubes develop nodules deeply imbedded in the agar, while in other tubes nodules either develop at the surface or else are given access in the air by shrinkage or cracking of the agar gel. Where the nodules are all deeply imbedded the plants remain weak, scarcely larger than uninoculated plants. Where the nodules develop with free access to the air, the plants grow much stronger (Plate 11, fig. 1). In the latter case there was appreciable nitrogen fixation, whereas the imbedded nodules produced no increase in nitrogen (Table II).

Table II. — Effect of Aeration of Nodules on Nitrogen Fixation in Lucerne Seedlings growing in Agar.

(Total nitrogen of seedlings plus agar medium.)

Tube number.	Total nitrogen.	Mean.
	mgs.	
Tubes with nodules exposed to air—		
1	4.22	} 3.53
2	4.13	
3	3.57	
4	3.12	
5	2.63	
Tubes with nodules imbedded in agar		
1	2.06	} 1.88
2	2.00	
3	1.90	
4	1.55	
Uninoculated tube	2.26	2.26

In certain cases nodules, at first deeply imbedded, were subsequently exposed to the air by shrinkage of the agar; such nodules then began to benefit the plant, producing a sudden increase in growth and darkening of the leaves (Plate 11, fig. 2). Sections were made of nodules deeply imbedded which had not benefited the host plant, and of nodules growing at the surface whose host plants were growing strongly. In neither case were the bacteria in the young nodule tissue attacking the host-cell contents or invading the cell walls. Thus unhealthy and stunted growth of the plant did not in itself cause the bacteria to become parasitic. They did not attack the host tissue, because their numbers were limited by lack of air and the carbohydrates brought to them supplied sufficient energy material. In healthy functioning nodules, it is probably the air supply which still limits the bacterial population. But where the carbohydrate supply is cut down, energy material becomes the limiting factor, and the bacteria consequently consume all available material in the host tissues.

The third condition in which the bacteria become parasitic upon the host tissues is that brought about in old nodules. Here their behaviour bears obvious resemblances to the change to active parasitism induced by shortage of carbohydrate supply. In both cases they destroy nuclei of the host cells and invade the cell walls from the infection threads. Here also the change in relationship between the bacteria and the host tissues is perhaps caused by a failure of the carbohydrate supply within the host cells, due either to lessened

photosynthesis as the season advances or to some failure in the translocation of carbohydrates into the nodule.

*Summary.*

1. When inoculated lucerne seedlings are placed in the dark, the formation of fresh nodules is soon stopped and nodules already formed soon cease to grow in size. This is associated with, and probably due to, the cessation of cell division throughout the root.

2. In such nodules, the bacteria become parasitic upon the host tissues, destroying the cytoplasm and nuclei of the infected cells, and invading the cell walls and intercellular spaces.

3. In old nodules on lucerne and clover plants growing in the light, the bacteria behave similarly. The swollen forms in the cytoplasm, after the destruction of the host nucleus, break up into granules which eventually disappear, while coccoid rod forms from the old infection threads invade the cell wall and intercellular spaces, causing the nodule tissue to disintegrate.

4. It is suggested that in both these cases the change in behaviour of the bacteria is due to lack of carbohydrate, and that, where this is limited, the bacteria derive their energy from the host tissue.

5. This hypothesis implies that, in normal nodule tissue, some other factor, perhaps air supply, limits the bacterial population and thus prevents them from attacking the host tissues.

6. Observations on lucerne seedlings growing in agar show that where the air supply is inadequate, the nodules do not function normally, although these conditions do not cause the bacteria to injure the nodule tissue, the carbohydrate supply not being the factor limiting their growth.

REFERENCES.

- (1) Stevens, J. W., 1925. 'Soil Sci.,' vol. 20, pp. 45-65.
- (2) Wright, W. H., 1925. 'Soil Sci.,' vol. 20, pp. 131-141.
- (3) Helz, G. E., Baldwin, I. L., and Fred, E. B., 1927. 'Journ. Agric. Res.,' vol. 35, pp. 1039-1055.
- (4) Mann, A. R., 1918. 'Cornell Univ. Agric. Exp. Sta. United States Dept. Agric. Rept.,' 1917, pp. 193-198.
- (5) Brenchley, W. E., and Thornton, H. G., 1925. 'Roy. Soc. Proc.,' Ser. B, vol. 98, pp. 373-399. ('Rothamsted Memoirs,' 1922-1925, vol. 12.)
- (6) Thornton, H. G., 1929. 'Roy. Soc. Proc.,' Ser. B, vol. 104, pp. 481-482. ('Rothamsted Memoirs,' 1927-1929, vol. 14.)
- (7) Bouin, P., 1897. 'Phénomènes cytologiques anormaux dans l'Histogénèse,' p. 19.
- (8) Milovidov, P., 1925. 'Publ. de la Faculté des Sci. de l'Univ. Charles, Prague,' vol. 49, pp. 3-40.
- (9) Prazmowski, A., 1890. 'Landwirt. Versuchs. Stat.,' vol. 37, pp. 161-238 (see Plate 1, fig. 15).

## DESCRIPTION OF PLATES.

## PLATE 7.

- FIG. 1.—Young lucerne nodule on normal seedling grown in the light, showing protoplasmic cells and large nuclei. Dividing cell (*m*), stele (*stl*), nodule cortex (*ct*). ( $\times 330$ .)
- FIG. 2.—Slightly older lucerne nodule on normal seedling, showing differentiation of tissues into meristem cap (*ms*), swollen cell region (*sw*), and nodule cortex (*ct*). ( $\times 200$ .)
- FIG. 3.—Nodule from lucerne seedling grown in the dark, showing disappearance of cytoplasm, shrinkage of nuclei and great increase in quantity of infection threads (*st*). ( $\times 330$ .)
- FIG. 4.—Part of the nodule shown in fig. 3 showing nuclei (*d*), being destroyed by bacterial zooglœa (*z*). Uninjured nuclei (*n*). ( $\times 1000$ .)

## PLATE 8.

- FIG. 1.—Swollen infected cells in a normal nodule from a lucerne seedling grown in light, showing large nuclei (*n*), and cytoplasmic strands (*cs*), uninjured although the cytoplasm is filled with bacteria. ( $\times 1000$ .)
- FIG. 2.—Swollen infected cells in a nodule from a lucerne seedling placed in the dark, showing destruction of the cytoplasm, and the shrunken and distorted nucleus (*n*). Masses of bacteria (*bac*), infection thread (*st*). ( $\times 1000$ .)
- FIG. 3.—Nodule tissue from lucerne seedling grown in the dark, showing invasion of the cell walls by the bacteria. ( $\times 1000$ .)
- FIG. 4.—Cells from the same nodule as fig. 3 showing infection of the cell wall (*cw*), from an infection thread (*st*). ( $\times 1000$ .)

## PLATE 9.

- FIG. 1.—Young swollen cell tissue from a nodule on lucerne grown in the light, showing bacteria in the banded rod stage. ( $\times 1000$ .)
- FIG. 2.—Swollen cell tissue from an old lucerne nodule (plant grown in light), showing that the bacteria in the cytoplasm have broken up into granules (*gn*), which gradually disappear (*gns*), while the cell walls have become infected with coccoid rods (*cw*). ( $\times 1000$ .)
- FIG. 3.—Tissue from a similar old lucerne nodule showing swelling of the infected cell walls (*cws*), and remains of cytoplasm (*gns*). ( $\times 1000$ .)
- FIG. 4.—Tissue from the centre of the same nodule showing collapse of weakened cell walls (*cws*). Hollow space in the centre of the nodule (*sp*), bacterial zooglœa (*bac*). ( $\times 1000$ .)

## PLATE 10.

- FIG. 1.—Young swollen cell tissue from clover nodule, showing "bacteroids" (*bac*). Host cell nuclei (*n*). ( $\times 1000$ .)
- FIG. 2.—Swollen cell from old clover nodule showing bacteria breaking up into granules. ( $\times 1000$ .)
- FIG. 3.—Swollen cell from old clover nodule showing bacteria from infection thread (*st*), invading the cell wall (*cw*). Remains of host cell nucleus (*n*). ( $\times 1000$ .)
- FIG. 4.—Tissue from old clover nodule showing invasion of cell walls. ( $\times 1000$ .)

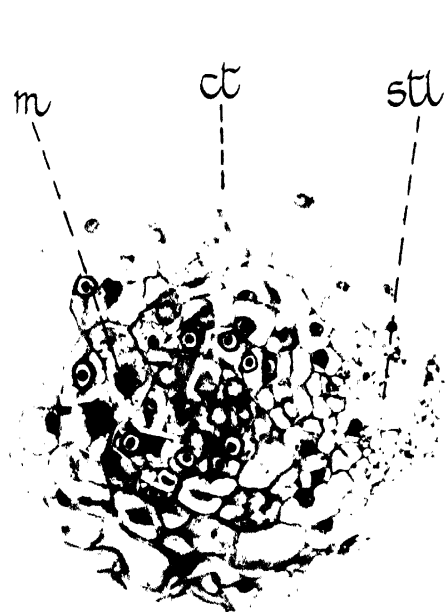


FIG. 1.

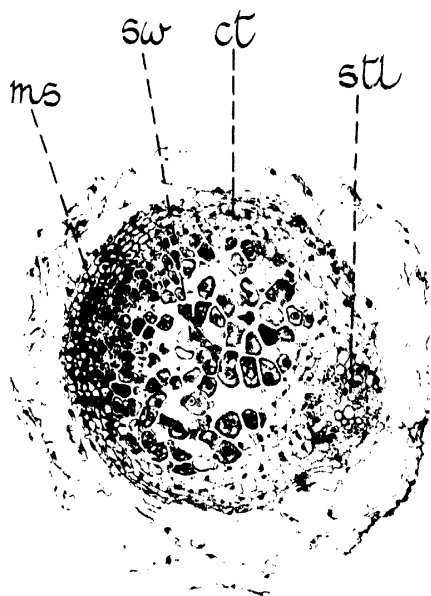


FIG. 2.



FIG. 3.



FIG. 4.



FIG. 1.



FIG. 2.

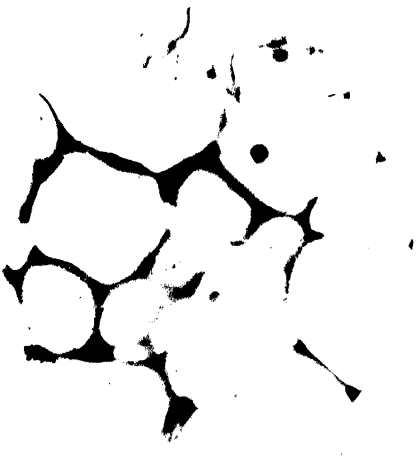


FIG. 3.



FIG. 4.



FIG. 1.



FIG. 2.



FIG. 3.

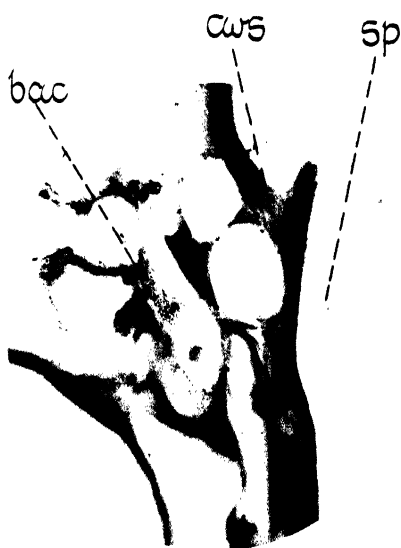


FIG. 4.



FIG. 1.



FIG. 2.



FIG.



FIG. 4.



FIG. 1.

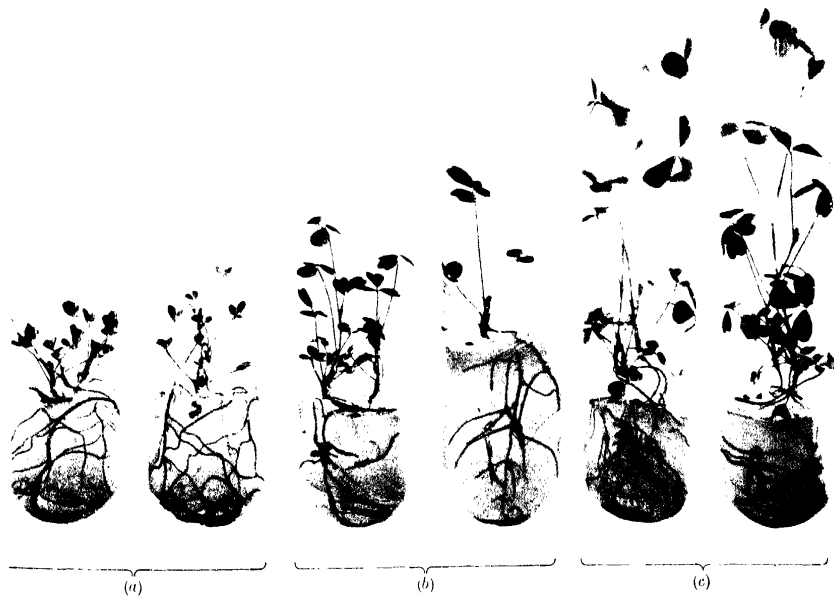


FIG. 2.



PLATE 11.

- FIG. 1.—Lucerne seedlings grown in agar. (a) Uninoculated controls, (b) Inoculated plants whose nodules are imbedded in agar, (c) Inoculated plants some of whose nodules are exposed to the air by shrinkage of the agar.
- FIG. 2.—Lucerne seedlings grown in agar. (a) Uninoculated controls, (b) Inoculated plants whose nodules were for some time imbedded but have since become exposed to air, (c) Inoculated plants some of whose nodules have been exposed to air from an early stage.



## THE INFLUENCE OF THE NUMBER OF NODULE BACTERIA APPLIED TO THE SEED UPON NODULE FORMATION IN LEGUMES.

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(With One Text-figure.)

It is natural to suppose that the greater the number of nodule bacteria in the soil surrounding their host legume, the better will be the chances of any given root hair becoming infected. The number of nodules formed should, in consequence, increase as the number of these bacteria, unless other factors limit the infection or subsequent nodule development.

This problem of the factors limiting infection has a bearing upon a related but less simple problem which is of importance to the practice of legume seed inoculation, namely: how far can the number of bacteria added to each seed be increased with a corresponding improvement in the number of nodules formed?

This question was investigated by A. T. Perkins<sup>(1)</sup>, who made experiments with Soybeans grown in sand cultures, and inoculated before sowing with varying numbers of nodule bacteria. He found that the number of nodules was increased when the number of bacteria per seed was raised from 1 to about 50, but that more bacteria than this did not produce more nodules. From this result he concludes that "after a certain degree of infection is reached the host is immune to further infection." The danger in drawing such a conclusion lies in the implied assumption that the number of bacteria added to the seed is similar, or at any rate proportional, to the number subsequently surrounding the root hairs. When inoculated seed is sown in soil or sterile sand the bacteria spread into the surrounding medium and increase up to a limit presumably set by the food supply or by competition with other organisms. This spreading and multiplication has been studied in sterilised soil by Thornton and Gangulee<sup>(2)</sup>. Perkins used sand containing nutrient salts but lacking in organic energy material which was presumably limited to substances produced from the plant's roots by secretion or by the decomposition of dead rootlets. Only 1 lb. of sand was used per

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pot containing 10 plants. Under these conditions it seems probable that the multiplication of the bacteria would soon be arrested by lack of energy material even when a small initial number of cells were introduced, so that a heavier inoculation would not produce a larger population surrounding the roots. In soil under field conditions, on the other hand, the plant's roots penetrate a larger volume of a substratum comparatively rich in energy material. The bacteria introduced on the inoculated seed will consequently be able to multiply to a much greater extent before their numbers are limited by lack of food material and until this limit is reached the number of cells originally added should affect the size of the population in the soil. Under field conditions, therefore, it was to be expected that the number of bacteria introduced on the seed should have an influence on the population of the bacteria surrounding the plant's roots even when large numbers were compared. Whether this difference in population would produce a difference in nodule numbers should be determined by the point at which the plant was able to resist further infection. There are thus two factors, either of which may set a limit to the effectiveness of increasing the dose of inoculum upon the seed, first, the maximum population of infecting bacteria attainable in the neighbourhood of the roots and secondly, the resistance to infection of the plant.

In actual farming practice another factor is introduced by the fact that the farmer is often unable to sow his seed immediately after inoculation. The consequent storage of the seed may therefore allow time for the death of many of the bacteria to take place. To approximate to practical conditions, therefore, the effect of varying concentrations of bacteria upon the seed must be tested both when the latter is sown at once and when it is stored for varying periods after inoculation and before sowing.

In the following field experiment the seed was inoculated at rates of one culture to 7, 14, 28 and 56 lb. of seed and, in order to test the power of the organisms to remain viable on the seed, each dose of inoculation was tried after storing the seed at room temperature for 1, 7, 14 and 28 days. The cultures used for inoculation were grown upon an agar medium containing extract of lucerne roots, mineral salts and saccharose, and the inoculation was carried out by means of a suspension of the bacteria in skim milk containing 0.1 per cent. of  $\text{CaH}_4(\text{PO}_4)_2 + 2\text{H}_2\text{O}$  (Thornton and Gangulee(2)). In making each inoculation, a suspension of the bacteria from four cultures was made and suitably diluted.

The trial was carried out on Colonel E. P. Brassey's estate at Upper Slaughter, Gloucestershire, the farming operations being supervised by Messrs C. Comely, of the County Education Office, and Mr W. H. Blake. The soil is shallow and calcareous, overlying flaggy limestone. It had been shown in a previous experiment on an adjacent field that uninoculated lucerne plants were practically free from nodules. The land used for the experiment had previously carried winter barley, folded off to sheep and had received no nitrogen other than that supplied by their droppings. The ground was on a slight slope and there was some gradient in fertility. To reduce the effects of this, each treatment was tested on duplicate plots and three were sown with uninoculated seed, the plots having the random arrangement shown in Table I, but with the two blocks placed in a single row. The total area of the 35 plots was  $1\frac{3}{4}$  acres. The seed was sown with hand drills on July 21 to 23, 1927, at the rate of about 18 lb. to the acre. The flow of seed through the drill was somewhat uneven. This seems to have influenced the subsequent growth.

Table I.

Block A																		
Pot No.	2	4	0	5	7	13	15	16	9	12	0	6	8	14	11	3	1	10
Lb. of seed treated per culture	56	56	Un-treated	28	28	7	7	7	14	14	Un-treated	28	28	7	14	56	56	14
No. of days the inoculated seed was stored	14	1	"	28	7	28	7	1	28	1	"	14	1	14	7	7	28	14
Block B																		
Pot No.	1	16	13	4	15	9	8	3	5	6	14	10	0	7	2	11	12	
Lb. of seed treated per culture	56	7	7	56	7	14	28	56	28	28	7	14	Un-treated	28	56	14	14	
No. of days the inoculated seed was stored	28	1	28	1	7	28	1	7	28	14	14	14	"	7	14	7	1	

The plant germinated well but during August it was attacked by millipedes and beetles and suffered a good deal of temporary damage. On September 24 sample plants were dug up, about 30 plants being taken from each of four different areas on each plot. These were taken to the laboratory, their roots washed out and the nodules on 50 plants from each plot were counted. The results of this count are shown in Table II. Increase in the strength of the inoculum has increased the nodule numbers up to the strongest dose used. But if the nodule numbers are plotted against the quantity of culture per lb. of seed, the curve rises steeply from uninoculated to the weakest inoculum tested and is flatter over the range covered by the experiment. The effect of increasing

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doses is thus less over this range than it would be at a weak concentration (Fig. 1).

The effect of storing the inoculated seed shows that the bacteria are remarkably resistant to drying on the seed. Seed stored for 14 days

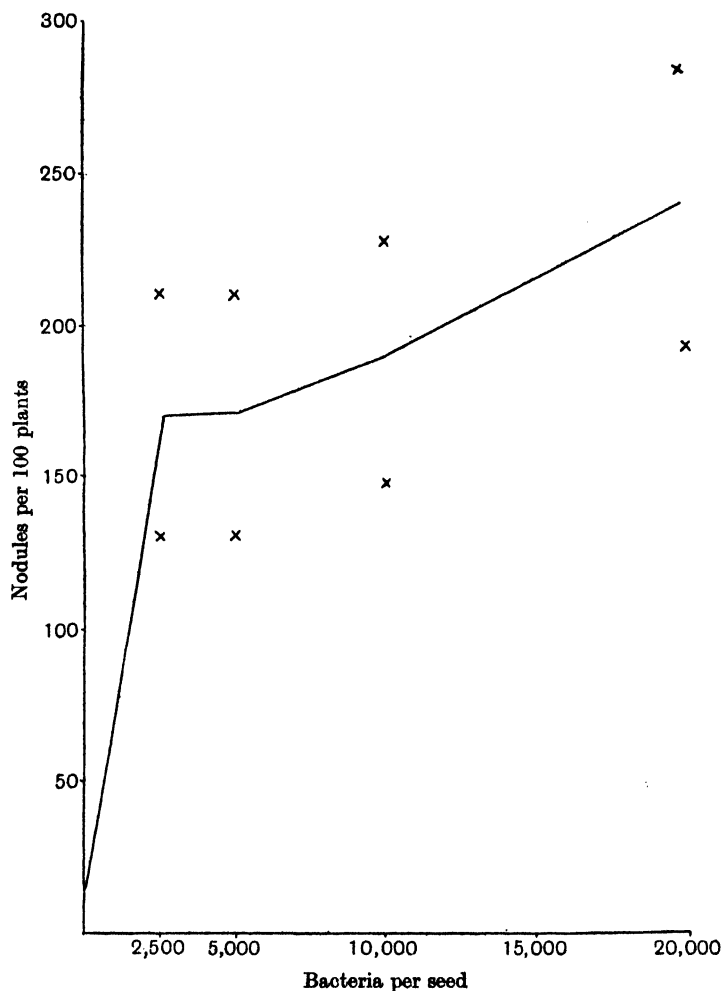


Fig. 1. Relation of nodule numbers to bacteria per seed.

The crosses represent the range covered by 3 times the standard error.

after inoculation still produced 61 per cent. of the number of nodules obtained from seed stored only 1 day. The effect of storage is greatest between 1 and 7 days. It seems therefore that the number of bacteria surviving upon the seed falls off most rapidly during the first few days.

The case may thus be compared with that of a suspension of bacteria or fungus spores in the presence of a killing agent (Henderson Smith (3)).

Table II. *Nodule Counts, Slaughter Field Trial, September 1927.*  
*Comparison of Parallel Plots.*

Period of storage (days)	1 culture to 7 lb. seed		1 culture to 14 lb. seed		1 culture to 28 lb. seed		1 culture to 56 lb. seed		
	Total nodules on 50 plants	X <sup>2</sup> of duplicate plots	Total nodules on 50 plants	X <sup>2</sup> of duplicate plots	Total nodules on 50 plants	X <sup>2</sup> of duplicate plots	Total nodules on 50 plants	X <sup>2</sup> of duplicate plots	
1	165, 176	0.355	154, 130	2.028	188, 119	15.508	79, 124	9.975	
7	102, 111	0.380	66, 82	1.730	53, 49	0.157	112, 117	0.109	
14	86, 121	5.918	100, 99	0.005	67, 73	0.257	67, 79	0.986	
28	98, 96	0.021	76, 50	5.365	97, 36	27.977	47, 55	0.627	
Total X <sup>2</sup>		6.674			9.128			43.899	11.697
(Total X <sup>2</sup> expectation 4.00.)									

*Comparison of Treatments.*

Period of storage (days)	Total nodules on 100 plants and standard error 7 lb. seed	Total nodules on 100 plants and standard error 14 lb. seed	Total nodules on 100 plants and standard error 28 lb. seed	Total nodules on 100 plants and standard error 56 lb. seed	Percentage of numbers obtained with fresh inoculum
1	341 ± 37	284 ± 34	307 ± 35	203 ± 28	—
7	213 ± 29	148 ± 24	102 ± 20	229 ± 30	61.75
14	207 ± 29	199 ± 28	140 ± 23	146 ± 24	61.00
28	194 ± 28	126 ± 22	133 ± 23	102 ± 20	48.9
Means	238.75 ± 15.4	189.25 ± 13.8	170.5 ± 13.1	170 ± 13.0	
Percentage of numbers obtained with strong inoculum		78.5	71.4	71.2	

*Notes.* The standard errors are based on the sum of the X<sup>2</sup> indices of the 16 sets of duplicates, and are actually calculated by taking twice the square root of each total count (cf. method developed by Fisher, Thornton and MacKenzie (4)) (3 times the standard error is a significant difference). 50 plants from each of the uninoculated plots bore 12, 6 and 4 nodules respectively.

In order to test whether the differences in nodule numbers, produced by varying concentration of culture and time of seed storage, were sufficient to affect the crop, weighings of the latter were taken. Table III shows the yield in lb. of green lucerne from 0.013 acre from each plot. The plots were very uneven as can be seen by comparing parallel plots. This seems to have been due to two causes. In the first place, the lower part of the field, bearing plots 9, 8, 3, 5, 6, 14, 10, 0, 7, 2, 11, 12 in Block B (Table I), had a much higher fertility than the remainder of the area. In the second place, uneven running of seed from the drills may have caused some plots to receive rather too little seed. The results of the weighings show, however, that there are no significant differences due either to amounts of culture or to length of time of storage in spite

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of the fact that inoculation has, on an average, increased the yield over untreated by about 100 per cent. The differences in nodule numbers found on the 2 months old inoculated plants have not been sufficient to affect the yield perceptibly.

Table III. *Upper Slaughter Lucerne Experiment.*

Result of weights taken from duplicate plots, September 14, 1928.

Period of storage (days)	Lb. of seed per culture				Means lb.
	7	14	28	56	
1	2000	2769	2538	1538	1961.25
	1769	1461	1769	1846	
7	1307	2884	2461	2846	1994.75
	1307	1538	1615	2000	
14	2654	2346	2692	2961	2345.75
	2000	1807	1807	2499	
28	1538	2807	2807	1615	1951.5
	1307	1692	1923	1923	
Means	1735.25	2163.0	2201.5	2153.5	2063.3
Untreated plots: 961, 691, 1384.			Mean of untreated plots: 1012.		

In the experiment above described, large numbers of bacteria were added per seed. A culture contains on an average 28,000,000,000 viable organisms. A pound of lucerne seed contains about 200,000 seeds, so that the number of organisms added per seed in the experiment ranged from 2,500 to 20,000. At these heavy concentrations a maximum effective dose was not reached under field conditions. In order to see whether a maximum effective inoculation could be reached by still heavier doses of the organisms, a pot experiment with Runner Beans (*Phaseolus multiflorus*) was made. In order to lessen the likelihood of the soil becoming saturated with the bacteria and thus affording a limiting factor, large pots were used containing 25 kg. of a mixture of equal parts Rothamsted unmanured soil and sand. This was maintained at a moisture content of 14.0 per cent., loss of water being determined by weighing each time the pots were watered. The following treatments were tested:

- A. Uninoculated.
- B. Seed inoculated at the rate of 1 culture to 16 lb. of seed.
- C. Seed inoculated at the rate of 1 culture to 4 lb. of seed.
- D. Seed inoculated at the rate of 1 culture to 0.5 lb. of seed.
- E. Seed inoculated at the rate of 1 culture to 0.5 lb. of seed and 4 oz. of chaff mixed into the soil + sand.
- F. Plants repeatedly watered with a thick suspension of the bacteria.
- G. Plants repeatedly watered with a thick suspension of the bacteria and half of their leaves removed.

The cultures used contained on an average 40,000,000,000 viable organisms, and the numbers added per pot on the inoculated seed were approximately 40,000,000 in series B, 160,000,000 in series C and 1,280,000,000 in series D. In series E, seed treated with the heaviest of these doses was sown in soil to which chaff had been added, it being known that the number of nodule organisms are thereby increased. In series F and G repeated doses of inoculum were added during the growth of the plants by pouring thick suspensions of the bacteria onto the soil<sup>1</sup>. In this manner about 150,000,000,000 organisms were added to each pot.

The numbers of nodules that developed in each series are shown in Table IV, in which the numbers per gram of root are calculated as this gives a better measure of the infection where the mass of the roots is variable.

Table IV. *Pot Experiment with Phaseolus.*

	Nodules per gram of root.						
	Block No.						
Series	1	2	3	4	5	6	Mean
A	83	81	52	108	69	55	78
B	84	64	98	130	114	111	100
C	95	64	90	53	100	50	75.3
D	94	164	119	95	136	218	137.3
E	165	156	224	315	91	116	178
F	234	329	284	284	288	—	284
G	215	390	219	450	199	154	271

The soil was not sterilised, since the establishment of the introduced organisms amongst the existing soil population was one of the factors to be considered. It contained a natural population of *Phaseolus* nodule bacteria, as is shown by the development of nodules on the uninoculated plants. The addition of 160,000,000 bacteria to the seed in series C has not increased the number of nodules but there is an increase produced by adding 1,280,000,000 in series D. The heavy dose needed to increase the nodules over control perhaps indicates a large population of *Phaseolus* bacteria in this soil. The increase is rather larger where chaff has been incorporated into the soil. In series F and G the nodules numbers have

<sup>1</sup> Six parallel pots were set up in each series, six seeds being sown per pot and the seedlings subsequently thinned out to three plants per pot. In series B, C and D the seed was inoculated by wetting with a suspension of bacteria in skim milk containing 0.1 per cent.  $\text{CaH}_4(\text{PO}_4)_2 + 2\text{H}_2\text{O}$ . The thickest suspension used was of such a strength that the milk just absorbed by  $\frac{1}{2}$  lb. of seed contained the bacteria from one culture. Weaker suspensions were obtained from this by dilution. The pots were arranged in the glasshouse in six blocks each containing one pot of each treatment. Within each block the pots were interchanged in position at intervals. The seeds were sown on August 15, 1927, and the experiment terminated on October 15. The roots were washed, the nodules counted and the dry weights of tops and roots obtained.

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been approximately doubled as compared with the heaviest seed inoculation in series D. In series G the removal of half the leaves caused a reduction in mean root weight to 1.45 gm. as compared with series F where the mean weight was 3.94 gm., but the number of nodules per gm. of root was not changed by the defoliation.

In this experiment, as in the field trial, no maximum effective dose of inoculum could be found. A comparison of this result with that obtained by Perkins<sup>(1)</sup> confirms the view that the effectiveness of his higher doses of inoculum was limited by the lack of sufficient food material in the sand to support any but a very small population of nodule bacteria. On the other hand, although the maximum effective dose was not passed in the experiments here discussed, the increases in nodule numbers have not been at all in proportion to the increased doses. Thus series D received about 1,280,000,000 organisms per pot and series F about 150,000,000,000, but this enormously increased dose has only doubled the number of nodules produced. The small effect of large doses of bacteria in increasing nodule numbers may be due to the population of nodule bacteria surrounding the roots being but little affected by the number originally added to the soil. Or it may result from a true immunity of the plant to heavy infections. The examination of root hairs of legume plants growing on agar seems to show that the plant cannot be infected by the organism to an indefinite extent. The following observations were made on young lucerne plants grown in wide tubes on agar under aseptic conditions and inoculated with a pure culture of the nodule organism. The absence of other bacteria from the tubes was checked by plating at the conclusion of the experiment. When the plants were about 6 weeks old the roots were examined and it was found that large numbers of the bacteria were clustered round the root hairs and that motile forms were also present. Nearly every root hair had a number of bacteria surrounding it. It was easy to see the infection threads entering the root hairs. In each of six plant roots, 100 root hairs, taken at random, were examined and the number found to contain infection threads in the six samples were 6, 3, 2, 8, 2 and 3. It would appear therefore that only a few of the root hairs actually become infected even where large numbers of the organisms are present amongst them. There would appear to be some factor other than the mere presence of the organisms that controls their entry into the root hairs.

## SUMMARY AND ABSTRACT.

In a field trial with lucerne grown from seed treated with varying doses of culture it was found that the numbers of nodules were increased as the dose was raised from 2,500 to 20,000 organisms per seed (56 to 7 lb. of seed per culture). Storing the seed for periods up to 28 days between inoculation and sowing, caused some loss in the nodule numbers. This loss was greatest between 1 and 7 days' storage.

The difference in dose of culture and in period of storage did not significantly affect the crop subsequently obtained from the inoculated plots, whose yield was, however, much above the uninoculated.

In a pot experiment made with runner beans, it was found that increase in the dose of culture above 1,280,000,000 organisms per pot containing six seeds was still capable of increasing nodule numbers but not to an extent proportional to the increase in dose.

The experiment does not exclude the possibility that the restriction in effect of very heavy doses may be due to the soil population becoming saturated with the bacteria. On the other hand, observations on lucerne plants grown aseptically on agar and inoculated with a pure culture, showed that even when excessive numbers of the bacteria immediately surrounded the root hairs, only 4 per cent. of these were infected.

## ACKNOWLEDGMENT.

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## REFERENCES.

- (1) PERKINS, A. T. *J. Agric. Res.* (1925), **30**, 95.
- (2) THORNTON, H. G. and GANGULEE, N. *Proc. Roy. Soc. Ser. B* (1926), **99**, 427.
- (3) HENDERSON SMITH, J. *Annals of Applied Biol.* (1921), **8**, 27.
- (4) FISHER, R. A., THORNTON, H. G. and MACKENZIE, W. A. *Annals of Applied Biol.* (1922), **9**, 325.

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## ON THE INFLUENCE OF THE CARBON : NITROGEN RATIOS OF ORGANIC MATERIAL ON THE MINERALISATION OF NITROGEN.

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(With Four Text-figures.)

### INTRODUCTION.

THE importance of the C : N ratio in biochemical soil processes has received considerable attention in recent years. Doryland (5) first stated definitely that the ammonification by *Bac. mycoides* and other saprophytic soil bacteria is governed by the proportion between the supplies of energy material and nitrogenous food, so that "beneficial" bacteria may become "detrimental" and *vice versa*. Hutchinson and Richards (8) considered especially the stabilisation of nitrogen in organic form during the fermentation of plant materials for the production of "artificial farmyard manure," and found a certain "nitrogen factor," expressing the quantity of N per unit of organic matter utilised by the micro-organisms which carry out the fermentation, and generally amounting to 2 per cent. of the dry organic matter. Waksman, on the other hand, considers especially the relationships in the soil and concludes theoretically (10) that organic materials with a N content of 2.0-2.5 per cent. will be decomposed with an immediate production of ammonia, whereas materials with less N will show a shorter or longer lag period or even nitrogen starvation, because all the N here is consumed by the micro-organisms which decompose the organic matter. Later on Waksman showed in collaboration with Heukelekian (11) that cellulose-decomposing fungi consume nitrogen in a definite ratio to the amount of cellulose decomposed, namely, one unit of N to every 30-33 units of cellulose. Anderson (1) also found that the most favourable N concentration for cellulose decomposition was 1 unit of N to every 35 units of cellulose, further supply of N being without effect. In quite recent work Waksman

<sup>1</sup> These experiments were started at the State Laboratory of Plant Culture, Lyngby, Denmark, where the soils were kept, and finished at Rothamsted Experimental Station during the writer's stay as a Fellow of the International Education Board. The writer is indebted to Mr H. F. Larsen for sampling the soils and forwarding the samples.

and Tenney<sup>(14)</sup> found plant materials to be decomposed in soil or sand without any extra consumption of N, if the materials contained at least 1.7 per cent. N. Little attention has been paid to these facts in relation to that important soil process, the mineralisation of farmyard manure, although it has long been well known that addition of straw to the soil tends to check nitrification and causes a more or less temporary decrease in fertility. A noteworthy series of papers on this subject has been published by Barthel and Bengtsson<sup>(2)</sup>, who in their last contribution found that when farmyard manure was added to three different soils no nitrification of the organic N took place in 14 months, whereas the ammonium-N was rapidly and completely nitrified. They sought to explain this by assuming that practically all the organic N was present in the form of bacterial bodies and epithelial cells—compounds which were not easily decomposed. In the present work farmyard manure was compared with a series of other organic materials of varying C : N ratio in order to see whether there exists in farmyard manure the same limiting C : N ratio as in other materials, and whether this limit is different in soils of different reaction, as might be expected, since it is generally assumed that fungi are, at least relatively, more active in acid soil, and since it is well known that fungi, when consuming a given quantity of carbon food, build up more protoplasm and consequently lock up more N than do the bacteria.

#### EXPERIMENTAL.

The materials used for the decomposition studies were the following: wheat straw (mature); sweet clover (*Melilotus alba*); blue lupin (*Lupinus perennis*); both these plants were cut at the time of full flowering; farmyard manure (pure, fresh cow dung, containing only a trace of ammonia); pea pods (green, of *Pisum sativum*); lucerne (*Medicago sativa*, young plants cut before flowering); and fungus mycelium (fruiting bodies of *Polyporus* sp. (*giganteus*?)). The contents of total carbon (determined by combustion according to Dennstedt<sup>(4)</sup>) and total nitrogen are given below.

Substance	In finely ground, dry material		
	% N	% C	C : N ratio
Wheat straw	0.54	about 45.0	(84 : 1)
Sweet clover	1.74	45.0	25.9 : 1
Blue lupin	2.26	45.2	20.0 : 1
Farmyard manure	2.33	sample lost at time of C determinations	
Pea pods	2.90	38.4	13.3 : 1
Lucerne	3.46	44.6	12.9 : 1
Fungus mycelium	4.45	45.5	10.2 : 1

Two soils were used for the experiment. One was a light sandy soil,

of strongly acid reaction and poor in organic matter, from an unlimed plot in a liming experiment at the Tylstrup Experimental Station, North Jutland; as little of the soil was available it was diluted with about 30 per cent. of pure quartz sand. This soil had been stored for a long time in an air-dry condition in the laboratory; to ensure the presence of living nitrifying bacteria it was inoculated, at the start of the experiment, with 0.2 per cent. of an acid, sandy forest soil in which nitrification was known to take place. The other soil was a light loamy garden soil of good fertility and faintly alkaline reaction. The soils were used in 600 gm. portions to which 12.0 gm. of the dry organic materials to be tested were added. The initial moisture content of the sandy soil was 12 per cent. and that of the loamy soil 20 per cent.; all the soil portions with additions of organic materials received further 2.0 per cent. water in order to saturate the increased water holding capacity to approximately the same degree as in the control soils. The soil portions were placed in 1500 c.c. Erlenmeyer flasks provided with tight fitting rubber stoppers through which passed a glass tube loosely filled with glass-wool. The flasks were incubated at 25° C. and for the first 15 days kept with the neck downwards in order to facilitate the escape of the CO<sub>2</sub> produced abundantly during the first stages of the decomposition. Samples of about 60 gm. of soil were then drawn at regular intervals, and determinations of NO<sub>3</sub>, NH<sub>4</sub> and reaction were made; while at the end of the experiment the humus was determined and estimations were made of nitrogen and methoxyl contained therein. Nitrate was determined by the ordinary Devarda method, ammonia by Bengtsson's<sup>(3)</sup> KCl method; humus was determined by the method devised by Waksman<sup>(12)</sup>, methoxyl by the ordinary Zeisel method. Reaction determinations were made at the start and for the first two periods by means of the quinhydrone electrode, and since then colorimetrically according to Gillespie. Only the initial and the final pH values are given here, the rest being of no special interest.

The results of the nitrate and ammonia determinations are given in Table I, and Figs. 1 and 2 show the amounts of inorganic N, as sum-totals of NO<sub>3</sub>N and NH<sub>4</sub>N, in acid and alkaline soil, respectively. The acid control soil is seen to show an excellent nitrification, in spite of its strongly acid reaction, but sweet clover and farmyard manure have both *depressed* the amounts of soluble N during the entire period. The pea meal shows some mineralisation of nitrogen, although of the 58 mgm. of N originally added, 42 mgm. remain as organic N after 6 months; and yet of the 2.9 per cent. N in the pea meal only 1.1 per cent., corresponding

## Mineralisation of Nitrogen

to 22 mgm. per 100 gm. of soil, is insoluble in hot water. The fungus mycelium shows an interesting effect: a considerable part of its N is ammonified during the first 2 months of the experiment, but is apparently consumed again during a later decomposition of some non-nitrogenous constituents of the mycelium, so that after 6 months the content of inorganic N is almost the same as in the control soil. In the alkaline

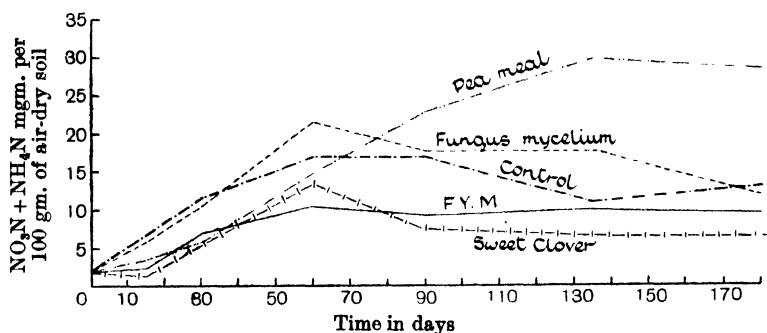


Fig. 1. Mineralisation of nitrogen in acid soil.

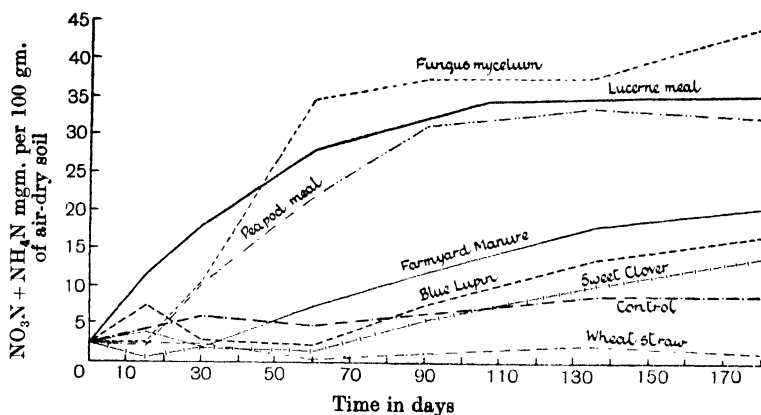


Fig. 2. Mineralisation of nitrogen in alkaline soil.

soil the results are more regular. The wheat straw with its very high C : N ratio gives rise to a constant depression of nitrate and ammonium content, although there is always a little mineral N present, a result supporting Anderson's observation(1), that nitrification and cellulose decomposition may occur simultaneously. The sweet clover has caused a smaller depression during the first 4 months; after 135 days this depression is overcome, and a definite increase in  $\text{NO}_3$  content is noted after 6 months. The lupin meal and the farmyard manure show rather

Table I.

I. *Production of  $NH_4$  and  $NO_3$  in acid sandy soil.*

pH of soil at start of experiment: 4.74.						
Time	1. Control		2. Sweet clover		3. Farmyard manure	
	$NO_3N$	$NH_4N$	$NO_3N$	$NH_4N$	$NO_3N$	$NH_4N$
Start	0.9	1.1	—	—	—	—
After 15 days	—	—	0.0	1.3	0.0	2.4
„ 30 „	10.9	0.7	4.9	0.4	5.9	1.4
„ 60 „	14.8	2.0	10.5	2.9	9.3	1.5
„ 90 „	16.5	0.3	7.4	0.0	9.1	0.0
„ 135 „	10.7	0.2	6.5	0.0	9.9	0.0
„ 180 „	12.4	0.4	6.4	0.0	8.8	0.6
Final pH	4.6		4.8		5.1	

Time	4. Pea pod meal		5. Fungus mycelium	
	$NO_3N$	$NH_4N$	$NO_3N$	$NH_4N$
Start	—	—	—	—
After 15 days	0.0	3.4	0.0	5.9
„ 30 „	2.8	2.9	0.0	10.6
„ 60 „	4.1	10.8	4.4	17.1
„ 90 „	7.5	15.1	6.3	11.3
„ 135 „	21.4	8.2	11.4	6.2
„ 180 „	19.0	9.1	9.2	2.6
Final pH	4.5		4.9	

II. *Production of  $NH_4$  and  $NO_3$  in alkaline loamy soil.*

pH of soil at start of experiment: 7.42.								
	1. Control		2. Wheat straw		3. Sweet clover		4. Blue lupin	
Time	NO <sub>3</sub> N	NH <sub>4</sub> N	NO <sub>3</sub> N	NH <sub>4</sub> N	NO <sub>3</sub> N	NH <sub>4</sub> N	NO <sub>3</sub> N	NH <sub>4</sub> N
Start	1.6	0.7	—	—	—	—	—	—
15 days	—	—	—	—	0.0	0.5	6.9	0.4
30 "	6.0	0.0	1.0	1.4	0.8	1.1	2.1	0.7
60 "	4.5	0.4	0.0	0.3	1.2	0.2	2.3	0.0
90 "	6.4	0.1	1.1	0.0	5.7	0.0	7.7	0.0
135 "	9.0	0.0	2.3	0.0	10.0	0.0	13.6	0.0
180 "	9.0	0.0	1.4	0.0	13.9	0.0	16.8	0.3
Final pH	6.8		7.0		6.8		6.9	

	5. Farmyard manure		6. Pea pod meal		7. Lucerne meal		8. Fungus mycelium	
Time	NO <sub>3</sub> N	NH <sub>4</sub> N	NO <sub>3</sub> N	NH <sub>4</sub> N	NO <sub>3</sub> N	NH <sub>4</sub> N	NO <sub>3</sub> N	NH <sub>4</sub> N
Start	—	—	—	—	—	—	—	—
15 days	3.5	0.4	0.0	2.2	0.0	11.3	0.0	2.5
30 "	0.8	1.0	8.6	1.5	7.4	10.4	0.3	9.9
60 "	7.0	0.3	21.7	0.7	27.6	0.2	34.1	0.4
90 "	11.9	0.0	31.1	0.1	—	—	37.0	0.2
105 "	—	—	—	—	34.4	0.0	—	—
135 "	17.9	0.0	33.6	0.0	—	—	37.3	0.1
150 "	—	—	—	—	35.2	0.0	—	—
180 "	20.6	0.3	32.3	0.2	35.2	0.0	44.3	0.0
Final pH	6.9		7.0		6.7		6.9	

more nitrification, the latter more than the former; unfortunately the carbon content of the manure could not be determined, but if a content of 42 per cent. is assumed as not unreasonable, we arrive at a C : N ratio of about 18 : 1. When we pass from this value to that of the pea meal, viz. 13.3, a large increase in the nitrate production is observed. The lucerne meal, in which the C : N ratio is only slightly lower than

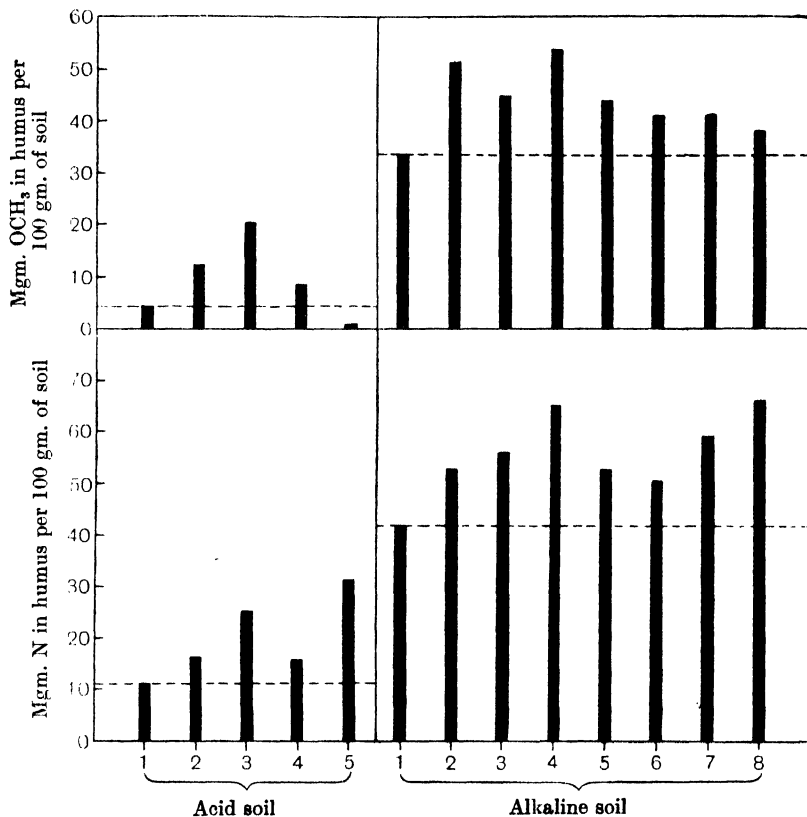


Fig. 3. Amounts of nitrogen and methoxyl in humus.

that of the pea meal, has shown a nitrification which is only slightly stronger, in spite of its considerably higher N content. Finally, the fungus mycelium with the most narrow C : N ratio behaves in an interesting manner: after a lag period there follows a very rapid production of mineral N which after 3 months comes almost to a standstill. There is thus a correlation between the C : N ratios and mineralisation of the nitrogen, the ratio of about 26 : 1 being about the limit above which no nitrification will begin within a period of 6 months in alkaline soil

under the conditions of the experiment; in the acid soil the limit lies much higher—somewhere between 18.1 and 13.3 : 1. It should be noted, however, that the time is a factor of considerable importance. In Fig. 3 the amounts of inorganic N in the alkaline soil after 1, 2, and 6 months are plotted against the contents of N in percentage of C. After 1 month there is a sharp break of the curve between the figures for pea and lucerne meal, but after 2 months the break is most pronounced between lupin and pea, and the former break is less marked. After 6 months the lag period for sweet clover has been overcome, and the two other breaks have almost disappeared. The reason why the fungus mycelium falls out of the range of the other substances is probably the presence of an undecomposable nitrogenous fraction, as mentioned below.

Further it is of interest to note that in the case of substances rich in N one part of the N is mineralised rapidly, another only very slowly, the  $\text{NO}_3$  figures becoming practically constant for pea and lucerne meal after 3–3½ months; the same holds true of fungus mycelium, although this curve is a little more irregular. If the increase in  $\text{NO}_3$  content over control is taken as an index of the amount of  $\text{NO}_3$  produced from the organic matter, it is possible to calculate the amount of unnitrified N by subtracting this excess from the total amount of N in the organic matter; this calculation shows that the residues of N after 6 months are fairly constant in comparison with the amounts originally added and correspond to 1.5–2.2 per cent. of the original material, increasing with decreasing C : N ratio:

Substance	N added mgm.	N un- nitrified	% N original	% N	
				Nitrified	Unnitrified
Sweet clover	34.8	29.9	1.74	0.24	1.50
Blue lupin	45.2	37.1	2.26	0.40	1.86
Manure	46.6	35.0	2.33	0.55	1.75
Pea pods	58.0	34.5	2.90	1.17	1.75
Lucerne	69.2	43.0	3.46	1.37	2.15
Fungus mycelium	89.0	44.7	4.45	2.21	2.24

To determine whether a part of this not readily nitrified N has passed into the soil humus the amounts of “ $\alpha$ -humus” (crude mixture of humic and hymatomelanic acid) were determined according to Waksman<sup>(12)</sup>; the determinations were carried out in triplicates, and 2 portions of humus were used for N determinations, 1 for methoxyl determination. Table II gives the results.

The acid soil contains only a little “ $\alpha$ -humus” which is remarkably poor in N and methoxyl; sweet clover gives a slight and farmyard manure a very marked increase in humus, and both materials, especially

the latter, increase the percentage of both N and methoxyl; pea pods have not given any significant increase in the actual amount of humus, but the percentages of N and methoxyl are also here considerably increased; the fungus mycelium has increased the humus content almost as much as the farmyard manure, but what is especially noteworthy is the extraordinary increase in N content and the reduction of the methoxyl content. The garden soil is much richer in humus which also contains more N and methoxyl. The wheat straw has given a considerable increase in amount of humus, a slight decrease in N content and a very distinct

Table II. *Amounts and composition of humus in soils.*

	Humus % in air-dry soil	N % in humus	OCH <sub>3</sub> % in humus
I. Acid soil:			
1. Control	0.49	2.43	0.86
2. Sweet clover	0.57	2.86	2.13
3. Farmyard manure	0.83	3.03	2.43
4. Pea pods	0.51	3.07	1.68
5. Fungus mycelium	0.72	4.37	0.11
II. Alkaline soil:			
1. Control	1.05	4.00	3.20
2. Wheat straw	1.36	3.89	3.73
3. Sweet clover	1.32	4.24	3.40
4. Blue lupin	1.63	4.00	3.27
5. Farmyard manure	1.36	3.85	3.22
6. Pea pods	1.24	4.08	3.31
7. Lucerne	1.29	4.58	3.19
8. Fungus mycelium	1.33	4.97	2.86

increase in methoxyl content—a fact which would indeed be expected from a substance rich in lignin. The sweet clover has here given a much greater increase in humus than in acid soil; the N content, too, is increased considerably, the methoxyl only a little. The blue lupin has given a very large increase in humus, with no significant changes in N and methoxyl percentages (although the actual *amounts* of N and methoxyl in humus are increased). The manure has given almost the same increase in humus and reduction of N content as the straw, but has not affected the methoxyl content. The pea pod meal, deficient in lignin, has given only a small increase in humus, with almost unaltered N and methoxyl percentages. The lucerne meal, also poor in lignin, has increased the humus only a little and left the methoxyl content unchanged, but has given a very marked increase in N content. Finally the fungus mycelium has given a moderate increase in humus, as in the acid soil a striking increase in N content and a decrease in methoxyl. The original mycelium contains a fraction, amounting to about 12 per

cent. of air-dry matter, which shows great similarity to the "humic acid" or  $\alpha$ -humus of the soil: an amorphous, dark brown substance, soluble in alkalis with intensely black colour and precipitated by acids as a voluminous, flocculent gel; in dry condition it contains 5.08 per cent. N, 55.4 per cent. C, and only a trace of methoxyl. Like the soil humus it is very resistant to the attack of soil microorganisms, so that its N is not readily nitrified in spite of its narrow C : N ratio (11:1); in an experiment—to be described in a later contribution—where 1 per cent. of the substance was added to a fertile, faintly alkaline garden soil, no increased nitrification over control occurred within 5 months.

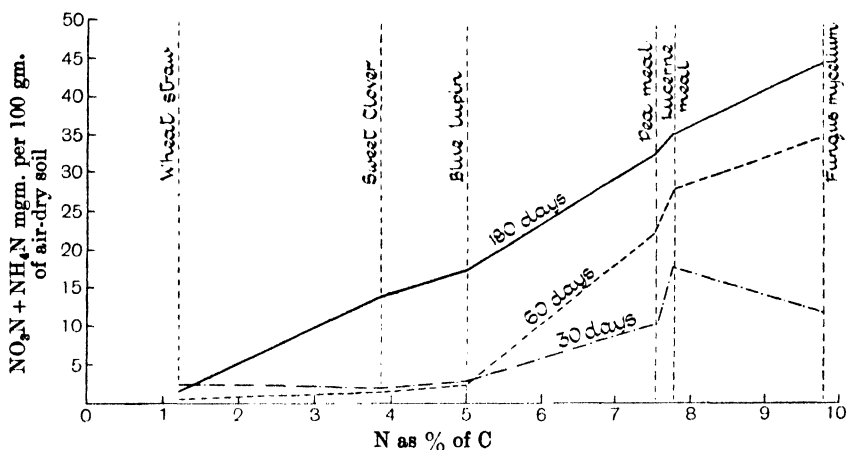


Fig. 4. Mineralisation of nitrogen in relation to composition of organic materials.

The amounts of nitrogen and methoxyl in humus in the differently treated soils are shown in Fig. 3. It seems that all the materials except the fungus mycelium have increased the humus content partly through their lignin content, partly in another manner which accounts for the increases in humic nitrogen. That lignin is a mother substance of soil humus can now, according to the studies of Fischer(7), Waksman(12), and du Toit(6), be regarded as settled beyond doubt; the origin of the nitrogen in the humus is somewhat more obscure; Waksman(12, 13) seeks the explanation for this in the synthesising actions of microorganisms, especially fungi which decompose cellulose actively in the soil and build up large amounts of protoplasm, and has actually shown(13) that a nitrogenous humus-like substance arises out of pure cellulose decomposing in pure sand with addition of mineral nutrients; the present results seem to lend some support to this theory when one considers the

increase in humus-N following the decomposition of such cellulose-rich substances as sweet clover and blue lupin, and lignin-poor substances as pea pod meal and young lucerne plants. Waksman<sup>(12)</sup> considers this nitrogenous humus a normal constituent of microbial protoplasm in general and fungus mycelium in particular; the present experiments furnish the evidence that *Polyporus* does indeed contain such a "humus"-fraction. But whether the formation of such substances is a general property of microorganisms can hardly yet be regarded as settled.

#### CONCLUSIONS.

The most striking result of the experiments is the fact that in general the C : N ratio of the added material influences the nitrification as much as does the soil reaction, and that in alkaline soil a certain part of the nitrogen added to the soil, namely one part of N for each 20–25 parts of C added along with the N, is nitrified only very slowly, whereas the excess over this is liberated quite readily. In acid soil the limit is much higher, viz. one part of N for each 13–18 parts of C, probably on account of the greater activity of the fungi which synthesise more protoplasm and consequently store up more nitrogen than the bacteria. The results thus conform well with those of Waksman and Tenney<sup>(14)</sup> and have a very interesting bearing upon the utilisation of the nitrogen in farmyard manure; the very slow nitrification of the last 1.5–2.0 per cent. of nitrogen is apparently the cause of the smaller effect of farmyard manure nitrogen in comparison with N as nitrate of soda, and the very rapidly decreasing nitrifiability which follows a decrease in N content towards 2 per cent. of the organic matter conforms well with the observation of Iversen<sup>(9)</sup>, that a loss of about 25 per cent. of the total N of farmyard manure implies a reduction of about 50 per cent. of the fertilising value of the manure in the first year; thus the nitrogen which is present in excess over what corresponds to a C : N ratio of 20 : 1 is the most valuable part and should be most carefully guarded against loss. Barthel and Bengtsson<sup>(2)</sup> found no nitrification of the organic N of farmyard manure (amounting to 2.12 per cent. of dry matter), but in this case the nitrification of the organic N of manure did occur, only 1.75 of the original 2.33 per cent. N being left unnitrified after 6 months. It should be remembered, however, that *fresh* manure was used, whereas Barthel and Bengtsson worked with old, well-rotted manure in which highly resistant nitrogenous compounds ("humus") may possibly have been formed during the fermentation.

Finally it is interesting to note that in the case of manure more of

the N has been found in the "humus" in the acid than in the alkaline soil, and in the former soil it should be still less available to the plants, because the humus of the acid soil has a lower N content than that of the alkaline soil and consequently is presumably still more slowly nitrified.

#### SUMMARY.

Organic materials with a C : N ratio ranging from about 85 : 1 to about 10 : 1 were submitted to nitrification tests in an acid and in an alkaline soil during a period of 6 months. In the acid soil only pea pod meal, with a C : N ratio of 13.3 : 1 showed an increase in inorganic N over control; in the alkaline soil the limit above which no nitrification will occur within a period of 6 months was at C : N = 26 : 1; below this limit the rate of nitrification increased rapidly with decreasing C : N ratio. Unnitrified N was left behind in a quantity corresponding to 1.5-2.2 per cent. of the original material, the percentage being higher in the case of materials rich in N.

All the materials tended to increase the content of " $\alpha$ -humus" in the soil, though not to the same extent or in the same manner. More " $\alpha$ -humus" was produced in the alkaline than in the acid soil, except in the case of farmyard manure. Straw, sweet clover, lupin and farmyard manure apparently acted both through their lignin content and through the synthesising action of microorganisms, since they increased the amounts of both N and methoxyl in humus. Mycelium of *Polyporus* contains a fraction possessing the properties of "humic acid," rich in N, but devoid of methoxyl, which persists in the soil.

As a general result the experiments show that the carbon : nitrogen ratio is a factor which exerts an influence on nitrification as profound as that of soil reaction, and that the less complete utilisation of farmyard manure nitrogen as compared with nitrogen in artificial fertilisers can to a large extent be explained hereby.

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### REFERENCES.

- (1) ANDERSON, J. A. *Soil Sci.* (1926), **21**, 115-126.
- (2) BARTHEL, C. and BENGTTSSON, N. Meddel. No. 311 fr. Centralanst. f. forsoksv. p. jordbruksomr. (1926). *Bakt. Avd.* no. 44.
- (3) BENGTTSSON, N. *Soil Sci.* (1924), **18**, 255-278.
- (4) DENNSTEDT, M. *Abderhalden's Handb. der Biol. Arbeitsmethoden*, Abt. I (1921), **3**, 485-503.
- (5) DORYLAND, C. J. T. *North Dakota Agric. Exp. Sta. Bull.* (1916), 116.
- (6) DU TOIT, M. S. Thesis, University of Cambridge (1924).
- (7) FISCHER, F. *Die Naturwissenschaften* (1921), **9**, 958-965.
- (8) HUTCHINSON, H. B. and RICHARDS, E. H. *J. Min. Agric.* (1921), **28**, 398.
- (9) IVERSEN, K. *Tidsskr. f. Planteavl.* (1927), **33**, 557-752.
- (10) WAKSMAN, S. A. *J. Agric. Sci.* (1924), **14**, 555-562.
- (11) WAKSMAN, S. A. and HEUKELEKIAN, O. *J. Biol. Chem.* (1925), **66**, 323-342.
- (12) ——— *Soil Sci.* (1926), **22**, 221-232, 323-333, 421-436.
- (13) ——— *Cellulosechemie* (1927), **8**, no. 9-10.
- (14) WAKSMAN, S. A. and TENNEY, F. G. *Soil Sci.* (1927), **24**, 317-333.

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# THE QUALITATIVE AND QUANTITATIVE EFFECTS OF FOOD ON THE GROWTH OF A SOIL AMOEBA (*HARTMANELLA HYALINA*)

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(With Three Text-figures.)

AMONG the free-living protozoa, excluding those forms which contain chlorophyll, two types of nutrition are found, holozoic and saprozoic; in the soil commonwealth there is an ample food supply for the saprozoic forms without reference to the other members of the micro-organic population, but the holozoic species present a different problem, since in the great majority of cases their food consists of the bacteria or other smaller organisms which occur with them in the soil solution. The idea that a normally holozoic animal might be induced to become saprozoic arose with the introduction of a suitable pure-culture technique. Among the earlier workers Beijerinck (1896), Frosch (1897) and Tsujitani (1898) all obtained "pure mixed cultures" of amoebae, and record their failure to grow these except in the presence of bacteria or yeasts, though Tsujitani was able to grow his amoebae with bacteria killed by heating them to 65° or 70° C.; later Mouton (1902) records that an amoeba, a form obtained from soil, would not grow except in the presence of living bacteria, and Oehler (1916, 1924), who has perhaps worked more in this field than any other observer, has experienced great difficulty in obtaining any protozoan growth in cultures which do not contain bacteria or yeasts, either dead or alive, though successful growth was obtained in the case of *Colpoda* by feeding the culture with ground-up fish or powdered egg albumen. It further appears from Oehler's work that, while the holozoic ciliates and flagellates are practically omnivorous provided that the food is supplied to them alive and of a suitable size, the amoebae tend to be very much more fastidious in their acceptance of food. All these writers seem to have been impressed by the enormous amount of bacterial food that a successful culture of protozoa can consume, and a quantitative study of this problem by Cutler and Crump (1924) showed that in the case of *Colpidium colpoda* in pure mixed culture the reproductive rate varied from 0.0 to 5.3 in 24 hours according as the number of bacteria per individual ciliate varied from 250 to 1,024,000; similar results have been obtained by Vieweger (1923)<sup>1</sup>, (1924).

Ever since the study of soil protozoa received its initial impetus from the work of Russell and Hutchinson (1909) this group of organisms, and more especially the

<sup>1</sup> Although the interesting work of T. Vieweger was published in 1923 it has only recently come to our notice.

amoebae, have been under suspicion of preying upon the bacteria and seriously reducing their numbers, thus constituting a possible menace to soil fertility. It has been established that in soil the numbers of active amoebae to a very marked extent vary inversely with those of the bacteria (Cutler, Crump and Sandon, 1922); further, that the numbers of protozoa present in any soil increase directly with the increase of organic matter (Sandon, 1927), in other words, with the crude supply of bacterial food. It seemed desirable therefore to get more direct information concerning the relationship of these soil amoebae to their food supply by a study of their behaviour in pure mixed culture. For this purpose a small linax amoeba *Hartmanella hyalina* was used, since it is a ubiquitous soil form and one of the dominant amoebae in normal field soils.

**Methods.** Pure mixed cultures of *Hartmanella hyalina* were obtained by the method which, with slight modifications, has been used by all earlier workers. A mixed culture of the amoeba is inoculated on to an agar plate either at one end of a streak of a pure culture of the desired bacteria or surrounded by a circle of them; after three or four days when the amoebae begin to appear at the other end of the streak or on the outside of the circle, a subculture is made in exactly the same way, and the process is repeated until the amoebae are obtained free from all but the requisite species of bacteria. The process can be facilitated and the number of contaminating bacteria reduced by washing cysts of the amoebae with 2 per cent. hydrochloric acid (sp. gr. 1.15) or some other suitable reagent for several hours. With certain bacteria, such as *B. prodigiosus* and *Azotobacter chroococcum*, we have been entirely unsuccessful; Oehler (1916) also records a failure to grow his amoebae with *B. prodigiosus* on bouillon agar owing to the production of trimethylamin, but states that they grow successfully together on a plain 1 per cent. or 2 per cent. agar. In the case of *Azotobacter* it seems probable that the size and consistency of the bacteria make it difficult for the amoebae to ingest them, though Cutler and Bal (1926) record the ingestion of dead *Azotobacter* by the amoebae.

The observations on the effect of food supply on *Colpidium* were carried out on mass cultures, but the same method is not applicable in the case of an amoeba, for it has been found impossible to obtain a good distribution of the animals in liquid culture owing to their tendency to crawl on the walls of the tube, concentrating in younger cultures just below the surface of the liquid in a band from which it is difficult to dislodge them. Single individuals were therefore isolated in ruled counting chambers covered with cover-glasses on which a drop of medium containing bacteria was suspended. The size of the drop was measured by the method detailed in an earlier paper (Cutler and Crump, 1923) and the amoebae could readily be counted under the low power of the microscope. In enumerating the bacteria, sample squares were counted and the assumption was made that the bacteria were uniformly distributed throughout the drop. If there was reason to believe that there was not this uniformity, even distribution was ensured by rotating the cover slip so as to mix the fluid before the bacteria were counted. The medium used in the experiments was soil extract prepared as follows: one kilogramme of soil was boiled for one-and-a-half hours with two litres of tap water and the mixture allowed

to settle, the supernatant fluid was poured off, made up to a litre, and filtered through two thicknesses of filter paper. The medium was then adjusted to a pH value of 7.2 and sterilised by autoclaving for 20 minutes at 115 lb. pressure. This medium has proved most successful for all the soil protozoa which have been grown in it; it is, however, open to the serious objection that repeated sterilisation renders it toxic, at least to *Hartmannella hyalina*. It is unsafe therefore to autoclave it more than once or at the most twice.

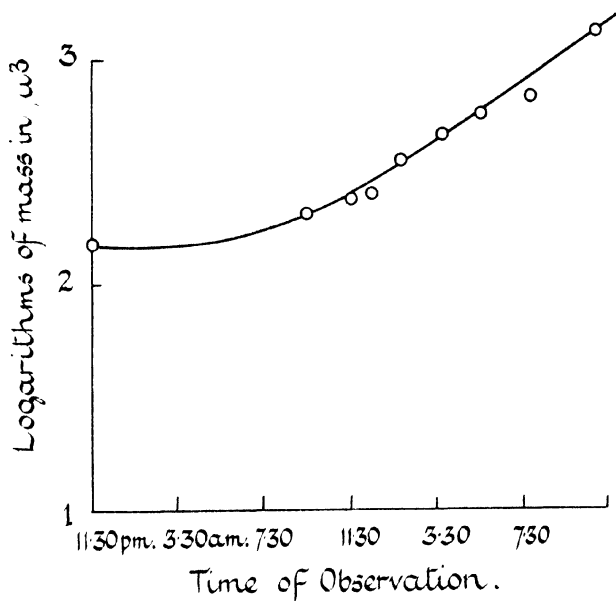


Fig. 1. Increase in mass of *Hartmannella hyalina* starting from a single specimen.

For the measurement of the actual increase in protoplasm as distinct from the increase in numbers, a camera lucida was used; ten drawings of the individual under observation being made as rapidly as possible on millimetre squared paper. The average number of squares in the ten outlines divided by the magnification gives the approximate area of the animal, and the thickness was regarded as  $1\mu$ . This method of measurement gives fairly consistent results (Fig. 1) but discrepancies may be found when the animal is stationary, as it tends to be thicker than when actively moving; in consequence the drawings were always made of moving animals. At a very early stage in the investigation it was obvious that the amoebae grew more readily with certain bacteria than with others; three species therefore were selected with which to obtain pure cultures, one giving extremely good growth, another poor growth, and a third—*B. mycoides*—which lay on the whole between the other two; all these species came originally from Rothamsted field soils.

#### *Description of bacteria.*

"YB." Rods,  $1.6-2.0\mu \times 0.8\mu$ , occasionally in pairs, non-motile, gram positive; colonies on nutrient agar—round, convex, buff, smooth shining, edge entire; on Thornton's

medium: round umbonate, white, smooth shining, edge entire; nutrient agar stab: nail-head, line of stab filiform; liquefies gelatine.

"SE." Short rods  $1.6 \times 0.8\mu$ , occasionally in pairs, non-motile, gram negative; colonies on nutrient agar: round convex, white, smooth shining, edge entire; on Thornton's medium: punctiform, white, convex, smooth shining, edge entire; nutrient agar stab: nailhead, line of stab filiform; does not liquefy gelatine.

*Experimental Results.* For purposes of comparison with the reproductive rates of the amoebae the numbers of bacteria may be calculated in two ways, either the number present per unit area may be made the basis of comparison or the total number present in the whole drop; in the first case the unit area being one square of the counting chamber, that is  $0.0025$  sq. mm. In the majority of cases either method of calculation would give the same results since in general the bacterial population is correlated with the size of the drop; but cases do occur where the total numbers of bacteria are the same but on the one hand the drop size is large and the bacteria are sparsely distributed and on the other hand the drop size is small and the bacteria densely distributed. Hence in the present work both methods of presentation have been used.

Table I. *Reproductive rates of Hartmanella hyalina with three species of bacteria.*

Reproductive rate of amoeba for 24 hours from one individual

No. of bacteria per square of $0.0025$ sq. mm. area	With "YB" bacteria		With <i>B. mycoides</i>		With "SE" bacteria	
	No. of cases	Average	No. of cases	Average	No. of cases	Average
0-12	12	2.7	19	1.8	8	0.6
12-24	32	3.3	55	1.6	43	0.8
24-36	37	3.8	49	2.1	44	1.6
36-48	67	3.7	36	2.4	44	2.0
48-60	63	3.9	25	2.6	22	2.0
60-72	44	4.2	6	2.4	18	2.4
72-84	22	4.5	—	—	12	2.6
84-96	19	5.0	—	—	6	3.0
96-108	12	4.9	—	—	1	3.3
108-120	4	4.9	—	—	—	—
120-132	4	5.1	—	—	—	—

A further reason for adopting both methods is that the question of food supply is not necessarily identical in each case: for there can be no doubt but that when there is a small drop densely populated the food is more accessible to a relatively slow moving organism such as an amoeba, than when the volume of the drop is big and the bacteria widely separated. Discrepancies due to this effect are eliminated by adopting the unit area as the basis of comparison, but by using this method the total available food supply is ignored. In every case the number of bacteria given is the average between the number present at the beginning and at the end of the 24-hour period. The results obtained with the three bacterial types (ignoring the drop size)

are shown in Table I, from which it is obvious that there is a definite correlation between the numbers of bacteria per unit area and the reproductive rate of the amoebae and, further, that the species "YB" induces considerably increased reproduction, the greatest rate in "SE" being only slightly greater than the lowest rate in "YB," though the sizes of the bacteria are nearly the same. *B. mycoides* lies intermediately as a source of food, but this species is hardly comparable with the other two, since it tends to form chains during its growth, and therefore one "mycoides" will not of necessity be equivalent to one "YB" or "SE." The size of the total bacterial population and its effect on the rate of reproduction of the amoebae is given in Table II, where a gradual increase in rate corresponds to an increasing food supply. These results are of course in effect the same as those given in Table I, but they are of use in showing the actual number of bacteria required by each amoeba in order to give any particular rate of reproduction.

Table II. *Reproductive rates of amoebae for 24 hours from one animal.*

Total bacteria per amoeba in thousands	"YB" bacteria		<i>B. mycoides</i>		"SE" bacteria	
	No. of cases	Average	No. of cases	Average	No. of cases	Average
0-200	9	3.2	31	1.7	10	0.7
200-400	25	3.4	44	1.9	24	1.4
400-600	21	4.1	41	2.1	29	1.4
600-800	19	4.4	21	2.2	26	1.6
800-1000	25	4.4	12	2.4	29	1.8
1000-1200	15	4.7	6	2.2	18	2.1
1200-1400	7	4.8	—	—	16	2.4
1400-1600	3	4.6	—	—	7	2.5
1600-1800	4	5.1	—	—	5	2.9
1800-2000	1	5.8	—	—	3	2.8
over 2000	4	4.6	—	—	3	2.3

It was thought possible, though not likely, that, although the rate of reproduction was markedly different in the "SE" and "YB" types of feeding, yet the total volume of amoebic protoplasm produced would not be very different. To test this possibility the experiments on the rate of growth of amoebae were carried out.

As is shown in Table III the "YB" bacteria gave a greater increase in volume than did the "SE." Unfortunately at the beginning of the experiment the numbers of bacteria in the "YB" culture were greater than in the "SE," while the amoeba in the "YB" culture was smaller than in the "SE" one. However from 3.45 p.m. onwards the bacterial populations were practically the same in the two cases, but the increase in the total volume of the amoebae present was 30 per cent. greater in the case of the "YB" culture.

The effect of feeding was also well shown by a series of observations recorded in Table IV. Two parallel cultures were started, one of which was well fed with "SE" bacteria, while the other had only the bacteria supplied with the isolation fluid and

was therefore in a relatively starved condition. This animal steadily decreased in size and after 24 hours it had shrunk to only  $83.5\mu^3$ . At this point food was introduced with the result that after five hours the animal had divided into five and the total volume had been more than six times increased. The well-fed amoeba on the other hand showed a steady increase in volume throughout the experiment.

Table III. *Growth of Hartmanella hyalina with different bacteria.*

Time of observation	Fed with "YB"			Fed with "SE"		
	No. of amoebae	Mass in $\mu^3$	No. of bacteria per 0.00025 mm. <sup>3</sup>	No. of amoebae	Mass in $\mu^3$	No. of bacteria per 0.00025 mm. <sup>3</sup>
Feb. 22						
10.15 a.m.	1	104.4	16	1	135.2	9
12.15 p.m.	1	104.4	21	1	124.0	10
2.15 p.m.	1	134.4	25	1	132.4	18
3.45 p.m.	1	168.8	27	1	143.6	23
5.45 p.m.	1	158.0	26	1	146.0	32
7.45 p.m.	1	182.8	36	2	178.8	39
Feb. 23						
7.45 a.m.	8	1496.0	56	5	758.8	62

Table IV. *The effect of starvation and feeding on mass in Hartmanella hyalina.*

Time of observation	Unfed		Fed	
	No. of amoebae	Mass in $\mu^3$	No. of amoebae	Mass in $\mu^3$
10.30 a.m.	1	248.7	1	159.4
11.30 "	1	246.0	1	185.7
12.30 p.m.	1	222.0	1	210.6
2 "	1	177.8	1	234.1
3 "	1	200.8	1	266.5
4 "	1	185.2	1	226.7
5 "	1	156.0	1	288.0
6 "	1	162.2	1	272.5
7 "	1	201.3	—	—
8 "	1	204.3	2	364.3
9 "	1	179.7	2	395.1
9.30 a.m.	1	143.4	5	895.2
* 10.30 "	1	83.5	—	—
11.30 "	1	159.2	5	901.6
12.30 p.m.	1	192.8	—	—
2.30 "	4	465.4	—	—
3.30 "	5	561.7	—	—

\* The culture was well fed after this observation was made.

Earlier work with the ciliate *Colpidium colpoda* gave a similar demonstration of the effect of food upon starved organisms. In Fig. 2 line drawings, each of 20 individual *Colpidia* taken at random are shown. The small organisms were taken from a starved culture, which on being fed contained within 24 hours animals similar to

the large ones seen in the drawing. Further the experiments on growth in mass show that the rate of growth is very variable after feeding, the starved animal having a much more rapid percentage increase in size per hour than is seen in the progress of a consistently well-fed animal. In comparing the effects of feeding either in respect to the species of bacterium used or to the quantity of food available it is found that the size at which the amoebae divide varies within wide limits, but is connected with both these conditions. For example amoebae well fed with "YB" bacteria on the average divide at  $274.0\mu^3$ : while those sparsely fed with the same species divide at  $191.6\mu^3$ . Using "SE" as the source of food well-fed animals divide at  $190.0\mu^3$ .



Fig. 2. Twenty individuals of *Colpidium colpoda* taken at random from a starved culture and from the same culture 24 hours later after heavy feeding with bacteria [x 250].

There are several records of differential feeding effects in amoebae and other protozoa. Frosch (1897) found that when he inoculated bacteria-free cysts of amoebae into pure cultures of various species of bacteria from soil he obtained very different degrees of growth. Tsujitani (1898) grew his amoebae successfully with a variety of bacteria, including *coli*, *fluorescens liquefaciens* and *non-liquefaciens*, *Staphylococcus pyogenes aureus*, *pyocyaneus*, typhus and cholera, but was unable to make them grow with anthrax bacilli or with yeasts. Mouton (1902) also found that *B. anthracis* gave poor cultures of amoebae, and records that *B. coli communis*, *Staphylococcus aureus*, the cholera bacillus and one or two other forms were suitable for his amoebae, as was a small yeast, *Saccharomyces exiguus*. Hargitt and Fray (1917), working with *Paramecium aurelia*, found that on the whole single species of bacteria did not give satisfactory cultures, while a mixed flora was successful, *B. subtilis* being the only bacterial species which gave good results in pure culture; Phillips (1922), working on the same lines and using three different bacteria, showed that alone these gave different degrees of growth, and in every case a mixture provided a more suitable food supply than did any single species.

Oehler (1916), in reviewing the subject, asserts that, on the whole, gram-negative bacteria are more readily eaten by amoebae than are the gram-positive species. Working with five distinct species of amoebae the following interesting feeding peculiarities were observed: two of his forms would eat *Saccharomyces exiguus*, while the other three would not; two of them would eat *coli*, *Sarcina* and

yeasts that had been autoclaved at 130° C. for an hour, while the other three scarcely grew at all under these conditions. One of these three would however readily accept *coli* killed at a temperature of 56° C. for one-and-a-half hours, and another would grow with *Bacterium fluorescens* killed by heating to 45° C. for one-and-a-half hours; the fifth species refused all these.

These different results obtained with different bacteria may be due to any of four causes: a species may be of a superior food value, or more suited to the digestive processes of the consumer, or possessed of toxic properties which inhibit growth, or of such a structure that for physical reasons it cannot readily be ingested. To decide between the first and second of these alternatives is by no means simple, but the third is obviously accessible to experimental attack. "SE" bacteria and *B. mycoides* were therefore grown in liquid cultures in soil extract medium, and on successive days 20 c.c. were taken from the mass cultures and filtered through porcelain candles; the filtrate was then diluted with an equal quantity of sterile, fresh soil extract and amoebae were isolated into the fluid in counting chambers in the usual way and fed with "YB" bacteria. The results of these experiments are given in Table V which shows that when extracts from a young culture are used there is no difference between the control and the treated cultures, but that the extracts from older cultures of "SE" bacteria have a bad effect. The same thing also occurs when extracts from an old culture of "YB" bacteria are used where no question of the bacteria having toxic properties can arise, therefore it appears that the inhibiting factor present in the older cultures of "SE" bacteria is due to extrinsic and not to any intrinsic properties of the bacteria themselves.

Table V. *Effect of bacterial extracts on reproduction of amoebae.*

Reproductive rates of amoebae for 24 hours all fed with "YB" bacteria

Control		Extract		Type of extract
No. of cases	Average	No. of cases	Average	
9	4.1	10	4.2	<i>B. mycoides</i> (2 days old)
12	3.8	12	3.7	" (3 days old)
15	5.0	14	4.9	"SE" bacteria (1 day old)
14	1.9	12	1.8	" (2 days old)*
12	2.2	13	1.9	" (3 days old)*
13	4.4	14	3.1	" (4 days old)
11	4.2	11	3.6	" (5 days old)

\* These cultures were incubated at 15° C. instead of 21° C.

Owing to the methods of counting employed in soil investigations it is difficult directly to apply the results outlined above to soil economy. Enumeration must be done in an indirect way and even when such a count is made it is incomplete since no one medium supports the whole bacterial population. Ammonifying species of bacteria predominate in these counts and many important species do not appear at all. Under these conditions it would not be likely that any direct ratio such as was

found in the fluid-culture experiments would emerge; but by employing only species of bacteria capable of growing on the culture medium used comparable results may be obtained. An experiment designed for other purposes, in which sand was inoculated in one case with "YB" bacteria and *Hartmanella* and in the other case with "SE" bacteria and *Hartmanella*, gave results which could be used in this connection. In Table VI the rates of reproduction of the amoebae are set out against the numbers of bacteria per amoeba. It is found that the rate of reproduction is consistently lower than it is in liquid cultures containing the same bacterial ratio, but that the correlation between reproductive rate and food supply still holds good, together with the lower food value of the "SE" bacteria.

Table VI. *Reproductive rates of Hartmanella hyalina in sand cultures.*

Successive days	Bacterial ratio	Reproductive rate
With "YB" bacteria		
1	1,800,000	3.0
2	124,000	0.5
3	34,000	0.0
4	190,000	0.5
5	127,000	0.0
6	250,000	0.0
7	260,000	2.5
With "SE" bacteria		
1	14,000,000	2.0
2	4,000,000	1.5
3	1,300,000	0.0
4	3,900,000	1.0
5	1,700,000	1.0
6	490,000	0.0
7	1,000,000	0.5

The lower rate of reproduction in soil or sand cultures would, however, be expected since the distribution of the food supply is such as to render it less available than in the liquid cultures used for the experiments. Here the bacteria are uniformly distributed and the whole of the liquid is accessible to the amoebae; in the soil on the other hand the bacteria are irregularly distributed through the film of water surrounding the soil particles and the amoeba is confined to the surface of the particles and not free to wander through the water film.

The figures in Table VI give some idea of the rapid changes that occur in the distribution of protoplasm among the constituent groups of the soil micro-organisms. For example, in the sand containing "YB" bacteria and amoebae, where on one day there are 7600 active amoebae, the 11,000 found on the next day represent a consumption of 1,444,000 bacteria. This figure is obtained by assuming that 0.5 division requires 190,000 bacteria per amoeba and therefore that 7600 amoebae will need the number of bacteria given above. An actual count of the bacterial numbers on the successive days showed a reduction of 1,134,000 bacteria, which is in keeping with the calculated result. When it is remembered that the bacteria are undergoing rapid divisions the large bacterial numbers required to keep up the protozoan population are not so unreasonable as might at first appear to be the case.

Where "SE" bacteria are the only available forms the number required to maintain the same sized amoebic populations as occur with "YB" bacteria is of course even greater. An increase of 400 amoebae to 600 (0.5 division) requires 480,000 bacteria; but if the numbers of amoebae had been 7600 as in the previous case, and had risen to 11,000, the bacterial consumption would have been 798,000,000,000. Interestingly enough, in our experience the numbers of amoebae have never risen above 900 when "SE" bacteria have been the sole source of food.

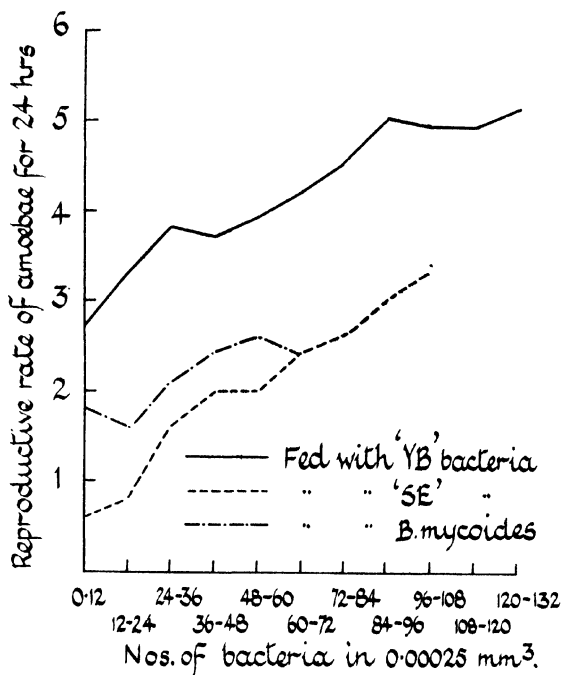


Fig. 3.

These results also have a bearing on the curious daily changes of the amoebic population which were recorded in a previous paper (Cutler, Crump and Sandon, 1922). It was found over a year's examination of soil samples taken daily from the field that both the numbers of bacteria and amoebae showed large fluctuations. The bacterial changes in numbers showed an inverse relationship with the amoebae, but the changes in the numbers of amoebae were inexplicable, as gross external conditions (rainfall, temperature, water content) taken alone had no effect upon them.

If, however, one assumes that the bacterial population undergoes changes similar to those found in plankton, in its constituent species as well as in numbers, an assumption for which there is much evidence—and that the feeding values of the various species are as different as is the case with the soil forms "YB" and "SE," then it must follow that the size of the amoebic population must also undergo rapid

changes. For when a species like "YB" is in the ascendancy the numbers of amoebae will rapidly increase, but a change to the "SE" type of bacteria will cause the death of many amoebae unless the numbers of bacteria increase to a figure higher than is associated with normal soils.

## SUMMARY.

1. A definite relationship between the reproductive rate of a soil amoeba *Hartmanella hyalina*, and the available bacterial food supply has been demonstrated.
2. It has been shown that three species of soil bacteria have different feeding values not only in respect to the rate of division of the amoeba, but also in respect to the total increase in the amount of protoplasm.
3. The bearing of these results on the soil economy is discussed.

## REFERENCES.

- BEIJERINCK, M. W. (1896). "Kulturversuche mit Amöben auf festen Substraten." *Centralbl. f. Bakt. Abt. 1*, **19**, 257-267.
- CUTLER, D. W. and BAL, D. V. (1926). "Influence of Protozoa on the process of nitrogen fixation by *Azotobacter Chroococcum*." *Ann. Applied Biol.* **13**, 516-534.
- CUTLER, D. W. and CRUMP, L. M. (1923). "The Rate of Reproduction in Artificial Culture of *Colpidium colpoda*. Part II." *Biochem. Journ.* **17**, 878-886.
- (1924). "The Rate of Reproduction in Artificial Culture of *Colpidium colpoda*. Part III." *Biochem. Journ.* **18**, 905-912.
- CUTLER, D. W., CRUMP, L. M. and SANDON, H. (1922). "A Quantitative Investigation of the Bacterial and Protozoan Population of the Soil." *Phil. Trans. Roy. Soc. B*, **211**, 317-350.
- FROSCH, P. (1897). "Zur Frage der Reinzucht der Amöben." *Centralbl. f. Bakt. Abt. 1*, **21**, 926-932.
- HARGITT, G. T. and FRAY, W. W. (1917). "The Growth of Paramoecium in Pure Cultures of Bacteria." *Journ. Exper. Zool.* **22**, 421-455.
- MOUTON, H. (1902). "Recherches sur la digestion chez les Amibes et sur leur diastase intracellulaire." *Ann. l'Inst. Past.* **16**, 457-509.
- OEHLER, R. (1916). "Amöbenzucht auf reinem Boden." *Arch. f. Prot.* **37**, 175-190.
- (1924). "Weitere Mitteilungen über gereinigte Amöben und Ciliatenzucht." *Arch. f. Prot.* **49**, 112-134.
- PHILLIPS, R. L. (1922). "The Growth of Paramoecium in Infusions of known Bacterial Content." *Journ. Exper. Zool.* **36**, 135-183.
- RUSSELL, E. J. and HUTCHINSON, H. B. (1909). "The Effect of Partial Sterilisation of Soil on the Production of Plant Food." *Journ. Agric. Sci.* **3**, 111-144.
- SANDON, H. (1927). *The Composition and Distribution of the Protozoan Fauna of the Soil*. Edinburgh: Oliver and Boyd.
- TSUJITANI, J. (1898). "Ueber die Reinkultur der Amöben." *Centralbl. f. Bakt. Abt. 1*, **24**, 666-670.
- VIEWEGER, J. (1924). "Recherches sur l'inanition de *Colpidium colpoda*." *Arch. d. Biologie*, **34**, 479-506.
- VIEWEGER, T. (1923). "Les Rapports entre le développement des Bactéries et des Protozoaires." *Journ. Physiol. et Path. Générale*, **21**, 700-703.



## A STUDY OF THE PROTOZOA OF SOME AMERICAN SOILS

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In 1916 Kopeloff, Lint and Coleman (7) concluded their excellent and exhaustive review of past work on soil protozoology with the remark that it was a science still in its infancy. Since then much has been done to place our knowledge of these organisms and of their activities in the soil on a firmer basis, but, in spite of this, considerable differences of opinion still exist on the fundamental question of their significance in the economy of the soil. On the one hand, data have been accumulating at Rothamsted demonstrating that protozoa occur in the local soils in considerably greater numbers than was formerly believed, and that they are capable of playing a significant rôle in soil processes (3, 4, 9). On the other hand, counts made by R. V. Allison (2) of protozoa in some samples of American soils gave very low figures, and caused him to admit that they "might be regarded, in a general way, as supporting the counter-protozoa theory." The samples he used, however, had been stored for some time before the counts were made and he therefore added that "until careful studies of freshly sampled soils are made . . . all conclusions drawn in this connection must be regarded as distinctly tentative."

With a view to reconciling, if possible, these rather conflicting points of view, a detailed examination of various American soils was made, the primary object being to determine to what extent the protozoa in them are either numerically or qualitatively comparable with those found in English soils.

The greater part of the work was done at the New Jersey Experiment Station where attention was directed mainly to the experimental agronomy plots (table 1). Counts of protozoa were made by Cutler's dilution method (3, 11), and bacteria, actinomycetes, and fungi were counted by the plate method using the media recommended by Waksman and Fred (12). The samples used were all composite ones from a series of 6-inch borings. In all the plots wide differ-

<sup>1</sup> Now in the department of general microbiology, Rothamsted Experimental Station.

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<sup>2</sup> This paper is part of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Rutgers University.

TABLE 1  
*Results of examination of soils from the Experiment Station Farm, New Brunswick, N. J.*

SOIL	TREATMENT	DATE	CROP* YIELD	TOTAL N	pH	H <sub>2</sub> O	BAC- TERIA	ACTI- NOMY- CES	FUNGI	FLAGELLATES	AMOEBAE	CILIATES	NEMA- TODES
			pounds	per cent		per cent	millions	millions					
7A	No manure, unlined†	September	3,881	0.074	5.3	8.0	2.8	0.8	89,000	121	165	0	0
7B	No manure, lined†	September	8,689	0.080	7.0	9.9	6.4	3.3	28,400	196	222	0	0
9B	Minerals NaNO <sub>3</sub> (16 pounds), lined†	October	18,599	0.074	6.8	11.0	4.7	2.7	12,184	770	938	25	0
5B	Complete minerals + cow manure (1600 pounds) annu- ally, lined	October† November January† March 2 March 25-28§	19,033	0.128	6.9	11.3 12.8 ..... 16.6	6.6 12.3 4.4 4.2	3.3 3.8 8.1 1.7 7.5	17,344 44,800 17,400 28,800 .....	719 <115,000 2,600 2,600 <222,000	1,010 28,770 13,600 44,000 125,000	15 <28,770 135 110 <186	0 0 0 Few 0
5A	Same as 5B, but unlined	January† March 2 March 25-28§	21,812	0.134	5.2	23.2 17.5	3.9 13.5 ....	7.7 8.4 5.4	46,400 177,200 .....	1,750 900 17,700	18,600 >220,000 46,600	104 160 172	Few 0 0
Penn loam	Alfalfa Fallow after corn	April§ April§	..... .....	..... .....	... ...	15.25 16.9	23.5 36.5	7.7 10.4	8,543 58,013	10,785 4,795	10,785 81,495	98 263	0 Few

\* Total dry matter 1918-1922.

† Average of 4.

‡ Average of 2.

§ Average of 3.

ences occurred in the numbers of all the groups of organisms at different times of the year similar to those found in the course of the Rothamsted counts of 1920-21 (3), where the changes have been shown to occur simultaneously in various parts of a field and therefore not to be due simply to sampling errors. Thus in plot 5B (receiving cow manure, and minerals annually, with occasional liming) the average of two counts made in October, 1925, was 700 flagellates, 1,000 amoebae, and 15 ciliates per gram, numbers which are slightly lower even than those obtained by Allison for the same soil. On the basis of these figures a series of dilutions was selected for making a count the next month. These were found to be totally inadequate, flagellates occurring in all the cultures, even those made from the highest dilutions. No estimate, therefore, could be made of their numbers except that they must have exceeded 115,000 per gram. The amoebae were about 28,770 and the number of ciliates, though the exact figures were uncertain, was even greater. At the same time the bacteria rose from 6.6 million to 12.3 million and the fungi from 17,300 to 44,800 whereas the actinomyces rose slightly from 3.3 to 3.8 million. The number of ciliates was most remarkable and was never again approached.

In view of the strange effects of the freezing of soil on its bacterial flora, which have been recorded from time to time, and the paucity of information as to the effect of frost on protozoal numbers, counts were made on this plot at intervals throughout the winter. The average of two counts made in January, 1926, at a time when the soil was frozen so hard that the sample had to be taken with a hammer and chisel, gave 2,600 flagellates, 13,600 amoebae, and 135 ciliates. All of these figures are considerably higher than those obtained in October. The number of fungi was practically unchanged (17,400), but that of the actinomyces was remarkable, being 8.1 millions per gram as compared with 4.4 million bacteria. This result is particularly unexpected since Lochhead (8) not only did not obtain any increase in the numbers of actinomyces in frozen soils at Ottawa, but showed further that their optimum temperature is higher than that of the majority of soil bacteria. It is possible therefore that the higher figures obtained in the present work are the result simply of greater fragmentation of the actinomyces and do not indicate any real increase in numbers. Waksman (10) gives no record of the numbers of actinomyces found in the course of his investigation of the seasonal changes in bacterial numbers in the New Jersey soils. Lochhead also recorded a slight depression in protozoal numbers during freezing, which again is contrary to what was found in the present investigation. Such differences may be due to the additional severity and length of the Canadian winter, but, in view of the wide margin of error in all counts of soil organisms, far more counts than have yet been made are required before we are justified in claiming finality for any conclusions on such matters.

On keeping one of these samples in the laboratory at room temperature for 3 days, big increases in numbers of all the organisms except the actinomyces occurred. Flagellates and amoebae both increased to more than 220,000 per

gram; ciliates to 2,280; bacteria to 15 million; and fungi to 46,400, whereas actinomyces fell to 5 million.

By the beginning of March the soil had thawed to a depth of a few inches and counts made of a sample of this thawed soil showed little change as compared with the completely frozen soil. This sample was afterwards dried in the laboratory for 24 hours at 32°C. and a second count then made, but even this treatment led to little alteration in protozoal numbers though the bacteria rose from 4.2 to 14.5 million and the actinomyces from 1.7 to 5.5 million.

Finally three counts were made at the end of March when the soil was completely thawed. The bacterial and fungal counts were unfortunately lost but all the groups of protozoa showed a big increase, the flagellates averaging something over 220,000; amoebae, 125,000; and the ciliates not fewer than 186 per gram.

These winter counts were paralleled with samples from the corresponding unlimed plot 5A with very similar results, except that the count made at the beginning of the thaw (March 2) yielded extraordinarily high numbers (> 220,000) of amoebae.

Thus, so far as it is safe to draw conclusions from so few observations, it appears that severe frost does not diminish the protozoal population, and subsequent thawing acts as a stimulus to multiplication.

A few counts were made of other plots. Plots 7A (unlimed) and 7B (limed) which have received no manure or fertilizer since 1908 were very poor in protozoa and on the average of 4 counts the limed plot showed a slight advantage in numbers of protozoa when compared with the unlimed plot, corresponding to its greater bacterial flora. Plot 9B, receiving complete artificial fertilizers (nitrogen as  $\text{NaNO}_3$ ) contained a fauna and flora differing very little from the corresponding plot 5B in which the nitrogen had been added in the form of farm-yard manure and in which consequently much organic matter was present. These counts were made in October and, as already mentioned, those from 5B proved exceptionally low; consequently it is not possible to say whether this close similarity would persist throughout the year.

With the exception of the counts made just after the thaw, all the figures obtained were considerably lower than those found in the corresponding plots at Rothamsted. This doubtless corresponds to the much lower bacterial figures. Samples taken of adjacent plots of a different type of soil (Penn loam) on the same farm, however, gave figures for both bacteria and protozoa of the same order as those found at Rothamsted.

In order to compare some soils where conditions differ more widely from those in England than do those in New Jersey, single counts were made of bacteria and protozoa in a number of soils from the neighborhood of the experiment station at Logan, Utah (table 2). In order to secure as many protozoal counts as possible within 6 weeks, an abbreviated series of dilutions was used, in which each soil suspension contained one-fourth instead of one-half the amount of soil in the preceding one. The number of cultures made was thus much reduced

at a sacrifice of accuracy, the significance of the result being only  $1/5$  (i.e. a count of 10,000 organisms indicating only that the actual number lies somewhere between 2,000 and 50,000).

The irrigated sugar beet plots of the experiment station farm (of which 4 were examined) yielded a fair number of protozoa. The plot receiving manure equivalent to 30 tons per acre annually, contained considerably more organisms than the unmanured plots, and though the plot receiving an even heavier dressing (40 tons) of manure showed slightly lower figures, the difference is not significant.

Two samples of dry farm soil were taken (one under wheat stubble and the other fallow). The moisture content was only about 3 per cent in each of these and the protozoa were apparently all encysted. Flagellates and ciliates were very few, but the amoebae reached several thousand per gram in each sample.

A number of samples were taken of alkaline soils both from the field and from pots. Numbers both of bacteria and of protozoa were low, and the amoebae were invariably more numerous than the flagellates. It seemed possible, however, that the organisms in such soils had become adapted to very abnormal conditions and that consequently the neutral nutrient agar used as culture medium for the counts was not suitable for their development. In three cases, therefore, a duplicate count was made using agar to which no nutrients or salts had been added but in which soil or soil extract from the same sample as that being counted was incorporated. In all three cases the counts obtained with this medium were very much greater than those obtained with the ordinary agar. The numbers obtained from such abnormal soils with ordinary agar, therefore, do not give a true account of the soil fauna, and even for more normal soils the soil agar is probably preferable, especially in view of the ease with which it can be prepared. François-Perey (6), working in France, also obtained higher counts with a soil extract medium than with a meat extract agar.

Thus it appears that some degree of protozoal activity occurs in all these soils. In the very dry soils activity apparently ceases, but doubtless is resumed after rain. In alkaline soils numbers are also much reduced, particularly where the principal salt is carbonate ("black alkali"). The numbers are, however, much greater than would be expected if the protozoa were incapable of any activity but were limited to cysts brought in by the wind. In view of the fact that in artificial media many of the soil protozoa can live at very high degrees of alkalinity it seems probable that a little activity persists in all these soils, at any rate after rain when the osmotic pressure is somewhat reduced and some amount of leaching has taken place from the surface.

The nitrogen content of most of these soils, however, was found to be exceptionally high (table 2). This suggests the presence of an amount of organic matter which would normally be expected to provide the nidus for a microflora and fauna far greater than that actually found. This is true not only of

TABLE 2  
Results of examination of soils from Cache Valley, Utah

SOIL NUMBER	CROP AND TREATMENT	TOTAL N	SALTS	pH	WATER	BAC- TERIA	ACTINO- MYCES	FLAGELLATES	AMOEBAE	CILIATES	NEMATODES
		per cent			per cent	millions	millions				
Greenville Farm											
1	Beet, irrigated, no manure	0.16	Carbonates constitute about 45 per cent of all these soils	About 8.0	5.2	.....	1.0	899	7,193	28	Few
2	Beet, irrigated, no manure	0.14		7.5-8.0	6.4	.....	0.9	450	3,596	225	0
3	Beet irrigated, 30 tons manure	0.23		7.5-8.0	7.2	.....	1.7	14,380	28,770	114	0
4	Beet, irrigated, 40 tons manure	0.33		7.5-7.8	8.0	.....	3.5	7,193	7,193	225	0
Dry wheat land											
Stubble Fallow	Wheat and fal- low on alter- nate years	0.108	.....	7.5-8.0	3.1	19.0	8.5	114	7,193	Few	0
		0.126	.....	7.5	3.0	18.9	5.5	450 (25 cysts)	16,000 (All encysted)	38	0
Alkaline field soils											
A1	Wheat	0.098	CO <sub>3</sub>	7.5-8.0	3.2	8.5	2.1	225	14,380	56	0
A2	Wheat	0.114	CO <sub>3</sub>	7.5-8.0	3.3	8.4	2.9	{ Few { (7,193)*	{ 28 { (115,000)*	{ Few { (Few)*	0
R	Pasture	0.33	CO <sub>3</sub>	8.8-9.0	13.3	10.4	6.0	225 { (1,798)*	1,798 { (14,386)*	28 { (450)*	0
B1	Pasture	0.05	CO <sub>3</sub> and Cl	About 9.0	7.2	4.9	0.1	71	57	28	0

B2	Pasture just being ploughed up	0.07	CO <sub>2</sub> and Cl	8.8-9.0	7.7	12.8	1.0	$\left\{ \begin{array}{l} 57 \\ (57) \end{array} \right\}^*$	$\left\{ \begin{array}{l} 900 \\ (3,596) \end{array} \right\}^*$	$\left\{ \begin{array}{l} 7 \\ (57) \end{array} \right\}^*$	$\left\{ \begin{array}{l} 0 \\ (\text{Few}) \end{array} \right\}^*$
<i>Alkaline pot soils</i>											
85	.....	.....	Mainly Cl	8.8-9.0	.....	3.4	0.1	34	40	0	0
86	.....	.....	Mainly Cl	About 8.0	.....	7.1	0.9	16	3,000	3.4	0
97	.....	.....	Mainly SO <sub>4</sub>	<8.8	.....	2.5	0	12.5	225	3.4	Few
98	.....	.....	Mainly SO <sub>4</sub>	<8.8	.....	2.0	0	22.4	225	6.8	0
104	.....	.....	Mainly CO <sub>2</sub>	<9.6	.....	0.1	0	0	160	12.5	0
110	.....	.....	Mainly CO <sub>2</sub>	About 7.2	.....	3.9	0	7.5	12.5	0	0

\* Counts made with soil extract agar.

the alkaline soils but also of the manured plots on Greenville Farm; consequently it appears probable that not only the salts, but other factors such as soil temperature and moisture, limit the microbiological development in this area.

The part played by the protozoa in the economy of the soil does not, however, depend only on the total number present but is probably considerably influenced also by the species which make up this total; for, scanty as is our knowledge of their physiology and nutrition, there is considerable evidence of the existence of wide differences in these respects not only between the three main classes (flagellates, rhizopods, and ciliates) but also between the different genera and species within these classes.

Thus the exceptionally high figures obtained in the November count of plot 5B at New Brunswick were due entirely to the great development of three species—the flagellate *Cercomonas* sp., a “limax” amoeba, and the ciliate *Colpoda steinii*—other forms being almost entirely absent. This doubtless points to very different conditions from those which occur in normal heavily manured soils in which large numbers of protozoa are associated with a rich variety of forms.

A valuable qualitative survey of the protozoa in New Jersey soils was published by Fellers and Allison in 1920 (5) of which the present results as to the nature of the organisms present in those soils are largely a confirmation.

On the whole, the fauna both of the New Brunswick and of the Utah soils resemble closely those of the Rothamsted soils. Unfavorable conditions seem to affect the flagellates more than the amoebae, since, although in soils where protozoa are abundant it is the flagellates which preponderate, among the soils examined in the present investigation it was found that, where the total population is small, the flagellates are generally exceeded by the amoebae.

The most interesting differences occurred in the amoebae. At the time of their investigation, Fellers and Allison found *Naegleria gruberi* (Schardinger) Wilson to be by far the most abundant of the soil amoebae. The same was true in the soils at Rothamsted in 1920–21, but more recently this species has been there almost completely replaced by another “limax” amoebae *Hartmanella hyalina* Alexeieff. Both at New Brunswick and at Logan, however, these forms were found to be outnumbered by others at present not fully identified. The most abundant species at New Brunswick were very similar in appearance and movements to *Hartmanella hyalina* though on the average slightly larger. On the addition of sterile water to a culture, however, flagellate forms are produced as in *Naegleria* but distinguished by the possession of only a single flagellum (pl. 1., figs. 1, 2). It accordingly belongs to the genus *Hyperamoeba* (see appendix).

One of the amoebae found commonly at Logan was at first mistaken for this, since both the active amoebic form and the cyst were indistinguishable. As all attempts to obtain a flagellate form failed, this species is provisionally referred to in table 3 as “sp. C.”

TABLE 3  
Occurrence of various flagellates, rhizopods and ciliates in the soils tested\*

**Flagellata**

<i>Cercomonas</i> sp.....	5A(9/9), 5B(13/13), 7A(5/5), 7B(5/5), 9B(2/2), P.a.(3/3), P.c.(3/3), 1, 2, 3, 4, D.f., A1, A2, R, 86
<i>Cercobodo agilis</i> Moroff.....	?5A(1/9)
<i>Cercobodo vibrans</i> Sandon.....	?5B(1/13), ?7A(1/5), 7B(1/5)
<i>Helkesimastix faecicola</i> Woodcock and Lapage.....	9B(1/2)
<i>Monosiga ovata</i> Kent.....	5B(2/13), P.a.(1/3), P.c.(1/3)
<i>Phalansterium solitarium</i> Sandon.....	5A(1/9), 5B(3/13), 7A(2/5), 7B(2/5), 9B(1/2), P.a.(1/3)
<i>Bodo edax</i> Klebs.....	7A(2/5), 7B(3/5)
<i>Bodo saltans</i> Ehrbg.....	5A(1/9), 5B(5/13), ?P.c.(1/3)
<i>Bodo</i> sp. (?).....	5A(3/9), 5B(8/13), 7A(3/5), 7B(1/5), P.a.(3/3), P.c.(3/3), B2
<i>Heteromita</i> spp.....	5A(7/9), 5B(7/13), 7A(5/5), 7B(4/5), 9B(2/2), P.a.(1/3), P.c.(3/3), 3, D.f., A1, A2, R, B1, B2, 85, 86, 97, 98, 110
<i>Phyllomitus</i> sp.....	?R, ?B2
<i>Spiromonas angusta</i> (Duj).....	5A(?2/4), 5B(?2/13), 7B(2/5)
<i>Sainouron mikroleron</i> Sandon.....	5A(1/9), 5B(6/13), 9B(2/2), P.a.(1/3), P.c.(2/3) 3, 4, R, B2
<i>Allantion tachyploon</i> Sandon.....	5A(5/9), 5B(7/13), 7A(1/5), P.c.(1/3), 1, 3, D.f., R, B1
<i>Proleptomonas faecicola</i> Woodcock.....	5A(4/9), 5B(7/13), 7B(1/5), 9B(1/2), P.c.(1/3)
<i>Spongomonas</i> sp.....	5A(4/9), 5B(1/13), P.c.(1/3), 3, 4, D.f.
<i>Tetramitus rostratus</i> Perty.....	5B(1/13), P.c.(?1/3), ?98
<i>Tetramitus spiralis</i> Goodey.....	5B(1/13), 7A(2/5), 7B(3/5)
<i>Oikomonas termo</i> (Ehrbg) Martin.....	5A(9/9), 5B(10/13), 7A(1/5), 9B(1/2), P.a.(3/3), P.c. (3/3) 1, 2, 3, 4, D.f., A2, R, B2
<i>Monas</i> sp.....	5A(1/9), R
<i>Scytomonas pusilla</i> Stein.....	5B(4/13), 7A(1/5), 7B(2/5), 9B(1/2), P.a.(1/3)
<i>Anisonema minus</i> Sandon.....	5A(1/9)
<i>Allas diplophysa</i> Sandon.....	5A(7/9), 5B(7/9), 9B(1/2), P.c.(2/3)
<i>Dimastigella trypaniformis</i> n. gen., n.sp.	5A(7/9), 5B(7/12), P.a.(3/3), P.c.(3/3), 3, 4
Unidentified spp.....	1, 2, 3, 4(2 spp.), D.st., D.f.(2 spp.), A1(2 spp.), R (3 spp.), B2

**Rhizopoda**

<i>Hartmanella hyalina</i> (Dangeard) Alexeieff.....	5A(8/9), 5B(12/13), 7A(5/5), 7B(5/5), 9B(2/2), P.a.(3/3), P.c.(3/3), 1, 2, 3, 4, R, B2, ?98
<i>Naegleria gruberi</i> (Schardinger) Wilson..	5A(4/9), 5B(5/13), P.a.(3/3), P.c.(3/3), A2
<i>Hyperamoeba</i> sp.....	5A(9/9), 5B(9/13), P.a.(3/3), P.c.(3/3)
<i>Amoeba limax</i> . sp. C.....	1, 2, 3, 4, D.st., D.f., A1, A2, R, B1, B2, 85, 86, 97, 98, 104, 110, (i.e., every Utah soil ex- amined)
<i>Amoeba limax</i> . sp. D.....	1, 2, 3, 4, D.st. D.F., A1, A2, R, B2, 85, 86, 97, 98, 104, 110, (i.e., every Utah soil except B1)
<i>Amoeba guttula</i> Duj.....	5B(?1/13), 1
<i>Amoeba verrucosa</i> Ehrbg.....	5B(?2/13), ?3, 4, A1, R

TABLE 3—continued

<i>Amoeba diploidea</i> Hartmann and Nägler	5A(1/9), 5B(6/13), 7A(2/5), P.c.(2/3)
<i>Amoeba striata</i> Penard.....	?P.a.(1/3), ?P.c.(1/3)
<i>Amoeba proteus</i> Pallas (Leidy).....	5B(2/13)
<i>Amoeba albida</i> Nägler.....	5B(2/13)
<i>Amoeba</i> "radiosa".....	3, R
<i>Amoeba</i> sp.....	5A(1/9), 5B(2/13), 7A(2/5)
<i>Biomyxa vagans</i> Leidy.....	5A(1/9), 5B(2/13), P.c.(1/3)
<i>Arachnula impatiens</i> Cienkowski.....	2
<i>Gephyramoeba delicatula</i> Goodey.....	5B(1/13)
<i>Nuclearia</i> sp.....	5A(1/9), 5B(4/13), P.a.(2/3), P.c.(2/3), 1, 3, 4, A1, R, B2
<i>Actinophrys</i> sp.....	5B(2/13)
<i>Diffugia globula</i> (Ehrbg).....	5B(1/13), R
<i>Euglypha</i> sp.....	5B(2/13), 4
<i>Trinema lineare</i> Penard.....	5A(2/9), 5B(2/13)
<i>Microgromia</i> sp.....	5B(5/13), P.a.(2/3), P.c.(3/3), 3
<i>Lecythium hyalinum</i> Hertwig and Lesser (= <i>Chlamydomorphys stercoreum</i> ).....	5B(2/13), 9B(1/2), P.a.(1/3), P.c.(1/3)
<b>Ciliata</b>	
<i>Holophrya</i> sp.....	5B(1/13), 1, 2, 3, 4, A2
<i>Enchelys</i> sp.....	P.c.(1/3), A2, R
<i>Prorodon</i> sp.....	R
<i>Chilodon</i> sp.....	5B(1/13), P.c.(1/3)
<i>Trochila</i> sp.....	?5A(1/9), 5B(1/13)
<i>Glaucoma</i> sp.....	?5B(1/13)
<i>Colpidium</i> sp.....	?5B(2/13), P.c.(2/3), ?R
<i>Colpoda cucullus</i> (O.F.M.).....	5A(8/9), 5B(7/13), P.a.(1/3), P.c.(3/3), 4, D.f., ?A1, A2, R
<i>Colpoda maupasii</i> Enriques.....	4, D.f., ?R, ?B2
<i>Colpoda steinii</i> Maupas.....	5A(8/9), 5B(12/13), 7B(1/5), 9B(1/2), P.a.(3/3) P.c.(3/3), 2, 3, 4, D.f., A1, R, B1, 98
<i>Cydidium</i> sp.....	A2, R
<i>Balantiophorus elongatus</i> Schewiakoff...	5A(6/9), 5B(11/13), P.a.(2/3), P.c.(3/3), 4
<i>Balantiophorus minutus</i> Schewiakoff...	?5B(1/13), 4
<i>Balantiophorus</i> sp. (probably either <i>B.</i> <i>elongatus</i> or <i>B. minutus</i> ).....	1, 2, 3, D.st., A1, B1, 86, 97, 98, 104
<i>Holotrichous</i> sp. (unidentified).....	5B(2/13)
<i>Halteria</i> sp.....	R
<i>Uroleptus</i> sp.....	5B(1/13), R
<i>Onychodromus</i> sp.....	?A2, ?R
<i>Gonostomum affine</i> (Stein).....	?1, R
<i>Oxytricha</i> sp.....	5B(1/13)
<i>Pleurotricha</i> sp.....	?5B(1/13)
<i>Hypotrichous</i> sp. (unidentified).....	5A(4/9), 5B(3/13), 3, 4
<i>Vorticella microstoma</i> Ehrbg.....	5B(1/13), P.st.(1/3), A2, R

\* The symbols following each name indicates the soils in which that organism was found. The symbols are the same as those used in the previous tables with the further abbreviations P.a. = Penn loam alfalfa, P.c. = Penn loam corn, D.st. = dry farm stubble, D.f. = dry farm fallow.

Where more than one sample was taken of any plot the number of samples in which the species was found is indicated by the fraction in parenthesis, thus *Monosiga ovata*, 5B(2/12) means that this organism was found in 2 out of 12 samples from plot 5B.

The other common species at Logan (referred to as "sp. D") also gave no flagellate form and had a smooth, thin-walled cyst like that of *Naegleria* but without any pores.

Among the flagellates the most striking feature was the relative scarcity of *Heteromita* sp. and *Oikomonas termo* (two of the most widely distributed and generally most numerous of all soil flagellates) in the New Brunswick soils. An interesting new flagellate *Dimastigamoeba trypaniformis* was found in both places (see appendix).

In brief, therefore, these investigations indicate that the protozoan fauna is very similar in all soils: the forms present are very similar even in widely differing soils and the numbers are roughly related to the bacterial numbers. In a soil in which the bacterial numbers (as counted by the plate method) are of the order of 5 millions per gram, the protozoa can usually be estimated in hundreds. Where the bacteria number 10 to 20 millions the protozoa can be reckoned in thousands or tens of thousands, whereas corresponding to bacterial counts of 40 or 50 millions, the protozoa may be expected to reach several hundred thousand per gram or even to exceed a million. These numbers are naturally very approximate but are probably minimal ones, for whatever errors there are in the dilution method of counting would tend to make the figure obtained too small rather than too large, and the surprising results obtained by using a soil medium in place of the ordinary nutrient agar in making the counts with the alkaline soils suggests that modifications in technique may reveal a larger population in all soils than has yet been found.

#### APPENDIX

*Hyperamoeba* sp. In appearance and movements the amoeba is very similar to *Hartmannella hyalina* Alexeieff, but on the average it is slightly larger than the latter species. The flagellate form (readily obtained by addition of fresh sterile water to a culture) is elongated or pear-shaped with the broader end behind (pl. 1. figs. 1, 2). The posterior end is very metabolic and coarsely vacuolated, whereas the anterior end is clear and contains an easily visible nucleus. There is a single contractile vacuole situated at the middle. The flagellum is inserted at the anterior end and is directed forward, the length being approximately equal to that of the body. It appears incapable of serving as a true swimming organ since the flagellate simply turns around slowly and jerkily on its long axis without moving forwards. Sometimes, however, it crawls along the surface of the microscope slide like a *Cercomonas* or *Mastigamoeba*. The cysts (pl. 1., fig. 3) resemble those of *Naegleria* rather than those of *Hartmannella* in possessing pores, but differ in having relatively thick and corrugated walls.

The only "limax" amoeba known which has a flagellate form with only a single flagellum is *Hyperamoeba flagellata* Alexeieff, recently found by Alexeieff (1) in horse manure from Finland. He gives no account of cyst-formation or of the cytology of the amoeba, and the present species was not examined under the conditions in which are obtained the characteristic forms by which alone *H. flagellata* can be identified. Consequently, pending a more detailed examination, the present species will be referred to simply as *Hyperamoeba* sp.

*Dimastigella trypaniformis*, nov. gen., nov. sp. This is a rather narrow, spindle-shaped organism closely resembling a *Trypanoplasma* in its general appearance and mode of motion. It is about  $12\mu$  long, possessing two flagella of which one is inserted at the anterior end and directed forwards, being about  $2/5$  to full body length, and the other being about twice as

long and directed backwards. This trailing flagellum adheres to the surface of the body and is occasionally thrown up for part or all of its length, forming the border of an "undulating membrane." The body is, however, generally circular in section and this thinning out to form a lateral membrane is not common. In swimming the active sinuous motion is very similar to that of a Trypanosome. A contractile vacuole is usually easily visible at the anterior end of the body.

On staining the flagellate, however, the "blepharoplast" or "kinetonucleus," which is generally regarded as diagnostic of the *Trypanosomaceae*, is found to be absent. Each flagellum ends in a minute granule, and when the contractile vacuole is dilated at the moment of fixing, it is seen to lie between the two granules, pushing them apart (pl. 1., figs. 4, 5). This species thus differs from the Cercomonads in which the basal granules are connected to the nuclear membrane. Werbitzky (13) has shown that under the influence of certain drugs the blepharoplast of *Trypanosoma brucei* is lost without the life or activities of the organisms being in any other respect noticeably affected. This raises some doubt, therefore, as to whether the structure may be regarded as absolutely diagnostic for the family, for it appears plausible that a structure which can be lost under artificial conditions may also be permanently lost in the course of natural evolution. For the present, therefore, it is proposed to include this flagellate in the family *Cryptobiaceae* of the *Protomastiginae* under the name of *Dimastigella trypaniformis* nov. gen., nov. sp., the genus being defined as free-living flagellates closely resembling the genus *Cryptobia* Leidy (*Trypanoplasma* Laveran et Mesnil) but possessing no blepharoplast.

The mode of nutrition has not been observed, but bacteria can often be observed lying within the plasma in stained preparations.

In older cultures, cysts are formed (pl. 1., fig. 6). These are spherical bodies, 4 to 5 $\mu$  in diameter. The wall is noticeably smooth, possessing no pore, and appears as a single contour around the plasmic contents, which are very uniform, containing no refringent granules or vacuoles and completely filling the cyst. When stained, they show a simple vesicular nucleus often with a minute granule lying against its membrane. In this condition also the similarity to the corresponding resting stage of the Trypanosomes, as figured by several authors, is quite close.

Though many preparations have been stained and examined, division stages have not been observed. Excellent cultures of this species were ultimately obtained, but it appears to be rather sensitive to cultural conditions (i.e. possibly to the presence of certain other organisms) and its occurrence on the plates used for counting was so erratic that no estimate of its abundance could be obtained. As, however, it frequently occurred in cultures made from rather high dilutions of soil, it probably sometimes attains considerable numbers in the New Brunswick soils. Though it has not been recorded by any other investigators, it is probably rather widespread. Dr. F. C. Holmes told the author that he has observed it in soil cultures at the Boyce Thompson Institute. The author also found it once in Utah, and has observed it in England in cultures from some straw compost, and from several different soils, although always in small numbers.

## REFERENCES

- (1) ALEXEIEFF, A. 1924 Notes sur quelques protistes coprocoles. *Arch. Protistenk.* 50: 41-48.
- (2) ALLISON, R. V. 1924 A note on the protozoan fauna of the soils of the United States. *Soil Sci.* 18: 339-352.
- (3) CUTLER, D. W., CRUMP, L. M., AND SANDON, H. 1921 A quantitative investigation of the bacterial and protozoan population of the soil with an account of the protozoan fauna. *Phil. Trans. Roy. Soc. (London) Ser. B.* 211: 317-350.
- (4) CUTLER, D. W., AND BAL, D. V. 1926 Influence of protozoa on the process of nitrogen fixation by *Azotobacter Chroococcum*. *Ann. Appl. Biol.* 13: 516-534.

- (5) FELLERS, C. R., AND ALLISON, F. E. 1920 The protozoan fauna of the soils of New Jersey. *Soil Sci.* 9: 1-25.
- (6) FRANÇOIS-PÉREY, J. 1925 Influence du milieu du culture sur les numerations des protozoaires du sol. *Compt. Rend. Acad. Sci.* 180: 315-317.
- (7) KOPELOFF, N., LINT, H. C., AND COLEMAN, D. A. 1916 A review of investigations in soil protozoa and soil sterilization. *Centbl. Bakt.* (II) 46: 28-74. Republished *Soil Sci.* (1917) 3: 197-289.
- (8) LOCHHEAD, A. G. 1924 Microbiological studies of frozen soil. *Trans. Roy. Soc. Canada* 18 (Sec. 5): 75-96.
- (9) SANDON, H. 1927 The Composition and Distribution of the Protozoan Fauna of the Soil. Edinburgh.
- (10) WAKSMAN, S. A. 1916 Bacterial numbers in soils, at different depths, and in different seasons of the year. *Soil Sci.* 1: 363-380.
- (11) WAKSMAN, S. A. 1927 Methoden der mikrobiologischen Bodenforschung. Abderhalden's Handbuch der biologischen Arbeitsmethoden. Berlin.
- (12) WAKSMAN, S. A., AND FRED, E. B. 1922 A tentative outline of the plate method for determining the number of microorganisms in the soil. *Soil Sci.* 14: 27, 28.
- (13) WERBITZSKI, F. W. 1910 Ueber blepharoblastose Trypanosomen. *Centbl. Bakt.* (I) 55: 303-315.

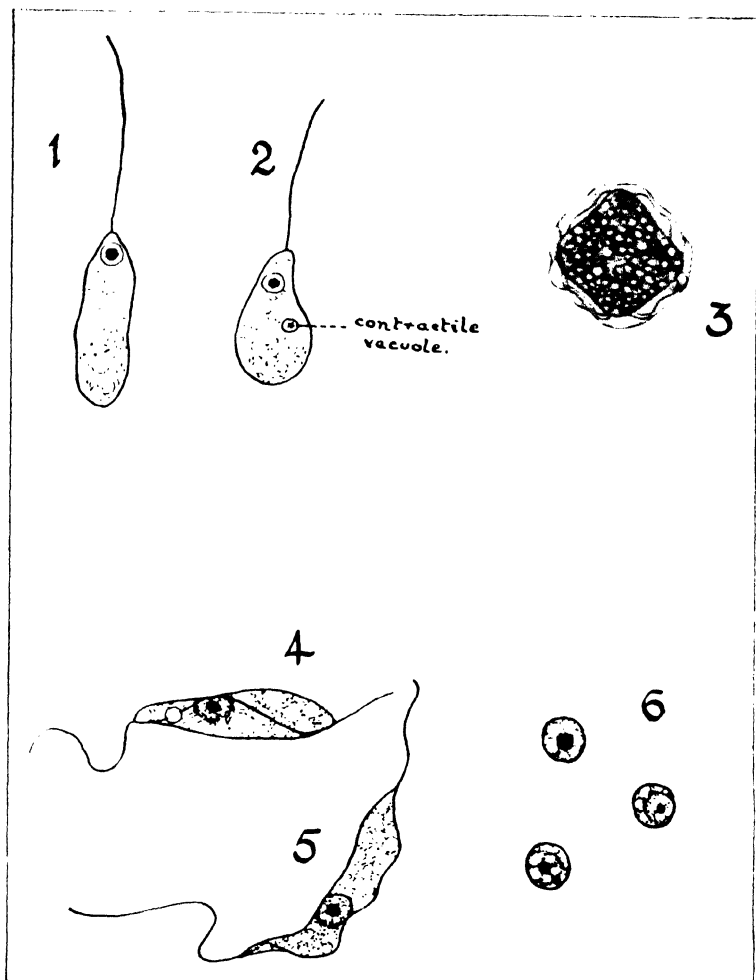
## PLATE 1

FIGS. 1 AND 2. *Hyperamæba* sp. Flagellate Stage. (Freehand sketches from life.)

FIG. 3. *Hyperamæba* sp. Cyst. (From life.)

FIGS. 4 AND 5. *Dimastigella trypaniformis*. Active Form. (Stained with Heidenhain's haematoxylin.)

FIG. 6. *Dimastigella trypaniformis*. Cysts. (Stained with Heidenhain's haematoxylin.)





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## THE EFFECT OF PHENOL, CARBON BISULPHIDE AND HEAT ON SOIL PROTOZOA

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### INTRODUCTION.

WAKSMAN AND STARKEY<sup>(11)</sup> found that when soil was treated with 1 per cent. carbon bisulphide the protozoa were absent after 14 days but reappeared at the end of 28 days. The disinfectant at first depressed the protozoa which later began to increase rapidly, reaching a maximum only after 90 days. When similar soil was reinoculated the rise in bacterial numbers was more rapid but this was followed by a rapid fall. The protozoa also were evident after 14 days. Russell and Hutchinson<sup>(7, 8)</sup> found that treating the soil with 0.5–1 per cent. carbon bisulphide produces an enormous increase in bacteria from 2–121 millions per gm. after a period of 74 days. Russell and Golding<sup>(6)</sup> in a previous paper gave similar results though the bacteria did not reach quite such high numbers. They believed that the vapour of carbon bisulphide has great power of penetrating the soil and of reaching organisms which other chemicals such as toluene leave untouched. Matthews<sup>(4)</sup> experimented with a large range of chemicals when working on the partial sterilisation of greenhouse soils and she found that the bacteria fluctuated, usually being reduced for the first few days and then rising to a maximum and gradually falling to normal. The whole process she says is much slower in field soil than in richer, lighter and better aerated greenhouse soil. Aeration she suggests has a great influence on the rapidity of these changes. She concluded that the rise in numbers of the bacteria is due not to the absence of the protozoa, but to the feeding effect of the antiseptic on the bacteria; and the increased fertility of the soil is to be attributed to the activity of the bacterial population in breaking down the organic matter of the soil. Sewertzoff<sup>(10)</sup>, working on the influence of several antiseptics on soil amoebae and bacteria, gives results quite the reverse to those quoted above; she finds with carbon bisulphide that even such high dilutions as 20, 40 and 60 per cent.

are useless to kill off the cysts of the soil protozoa or the spores of *Bacillus subtilis*. Even a non-sporing bacterium as *Staphylococcus* was not killed with 10 per cent. carbon bisulphide. It must be remembered that though these high dilutions are not strong enough to kill off cystic protozoa, the active stages are readily killed.

Buddin<sup>(1)</sup>, who worked at the partial sterilisation of soils by several antiseptics, found that phenol and its derivatives were effective if used in high enough strengths. He found that phenol in weak doses from  $M/200$ – $M/50$  causes a high rise in the numbers of bacteria, and that even up to 0.1 per cent. phenol there was no disappearance of the protozoa. Doses of  $M/10$  to  $M$  kept the soil protozoa and bacteria in an inactive condition for 75 days. Buddin did not use carbon bisulphide in any of his experiments.

In view of these discrepant results it was decided to carry out experiments on the effects of phenol and carbon bisulphide on both the cysts and active forms of known species of protozoa. Further, as steam is extensively used as a partial sterilising agent in glass-house work and since its action may not be the same on the soil population as that of volatile antiseptics its effects were also tested. The species of soil protozoa used were: *Naegleria gruberi*, *Hartmanella hyalina*, *Oikomonas termo* and *Cercomonas crassicauda*.

#### METHODS.

The cysts of the protozoa used were tested in two ways to discover whether they were killed or not by the treatment with the disinfectant. First by cultural methods, *i.e.* by placing the cysts on agar plates or in hay infusion. Second by testing with 0.125 per cent. eosin.

This method has been used by Wenyon, O'Connor and Cutler<sup>(3)</sup> as a rapid method of detecting dead cysts of *Entamoeba histolytica*, where it was of great use.

Cutler<sup>(2)</sup> has also used it for soil protozoa, the cysts of which were boiled or heated at 85° C. for one hour. These cysts, when tested with the watery solution of eosin, became uniformly coloured, and when tested by cultural methods were found to be dead. Kessel<sup>(3)</sup>, using this method for testing the viability of *Hartmanella hyalina* cysts when treated with chlorin water, finds also that all red cysts are incapable of development. Notwithstanding this the number of stained cysts does not in all cases represent the numbers of cysts which are killed. Frequently some of the cysts exhibit a condition of plasmolysis and have a yellow appearance. When the plasmolysis is complete, *i.e.* the cytoplasm

undergoes a very pronounced shrinkage, the cysts are then less likely to take the eosin stain. This complete plasmolysis was noticed occurring most commonly in high concentrations of chlorin water or in lower concentrations for extended periods of time.

Tests with *Hartmanella hyalina*, *Naegleria gruberi*, *Cercomonas crassicauda* and *Oikomonas termo* showed that the eosin method was effective with them, but with the flagellates *Helkesimastix faecicola*, *Heteromita globosa* and *Sainouron mikroteron* the dead cells were not coloured; in the case of the ciliates, *Colpoda steinii* and *C. cucullus*, the young or thin-walled cysts were coloured, but not all the older cysts with thick yellow and brown walls.

The eosin method is a useful rough test for the viability of cysts, but should be used in conjunction with other methods, and for some species of flagellates it is of no use. In all the following experiments, therefore, where the eosin method was used, the cysts were also plated on agar or placed in hay infusion to note whether excystation took place.

#### THE EFFECT OF PHENOL AND CARBON BISULPHIDE.

The following experiments were carried out to discover the strengths of phenol needed for destroying the active and cystic stages of the amoeba *Hartmanella hyalina* and two flagellates, *Cercomonas crassicauda* and *Oikomonas termo*.

##### *Experiments 1.*

Hay infusions containing the following strengths of phenol were used, 1, 0.5, 0.3, 0.15, 0.1, 0.055 per cent. and control.

*Method.* Cavity slides were filled with phenol hay infusion and active *Hartmanella hyalina* from a healthy rich culture were added. Evaporation was prevented by the slide being covered with blotting paper the central space being cut out, the paper was kept saturated with phenol hay infusion and placed in a damp chamber.

The amoebae in the dilutions from 0.1–1 per cent. were all killed; a few, rounded off with an unhealthy appearance, were found in the 0.055 per cent. dilution. The amoebae in the control were active and healthy. Another experiment was done in which active *Cercomonas crassicauda* were used instead of active amoebae. These were killed in every strength of the phenol while they remained healthy and active in the control.

*Experiments 2.*

The above experiments were followed by others in which cystic protozoa were used. As the form and results of the experiments are similar except for the protozoa used, it is needless to detail each in turn.

A suspension of cysts from an agar slope was made in 5 c.c. of sterile tap water, which was poured on to agar plates. The agar had previously been phenolised to the following strengths 1, 0.5, 0.3, 0.15, 0.1, 0.055 per cent. in duplicate and duplicate normal agar plates were used as a control.

The following cystic protozoa were used in different experiments: *Cercomonas crassicauda*, *Oikomonas termo* and *Naegleria gruberi*. In every case no excystation occurred in the dilutions above 0.3 per cent., though the cysts were not killed in the case of *Naegleria*, Table I; this result was doubly tested in the case of *Naegleria gruberi* as phenol hay infusion of the same strengths was used as well as the phenol agar.

Table I.

	1 %	0.5 %	0.3 %	0.15 %	0.1 %	0.055 %	Control
<i>Naegleria gruberi</i> cysts plated on phenol agar.							
Days							
1	+	+	+	+	+	+	a +
3	+	+	+	a +	a +	a +	a +
4	+	+	+	a +	a +	a	a
7	+	+	+	a	a	a	a +
17	v f +	v f +	+	+	+	+	a +
<i>Oikomonas termo</i> cysts in phenol hay infusion.							
2	+	+	+	a	a	a	a
5	+	+	+	f a	a	a	a
8	0	0	0	f a	a	a	a +
<i>Cercomonas crassicauda</i> cysts in phenol hay infusion.							
1	0	+	+	+	+	+	a +
4	0	+	+	+	+	a +	a +
7	0	+	+	a +	a +	a +	a +
10	0	+	+	a +	a +	a +	a +

v f = very few, f = few, a = actives, + = live cysts, 0 = dead cysts or actives.

These results were again tested by two methods to discover whether the cysts were killed by the phenol: (1) by the eosin method, where the dead cells were stained pink; (2) by plating on normal agar plates and examining these at intervals of three days to note whether any active protozoa were present. *Naegleria gruberi* cysts under these tests gave no excystation on the phenol agar after 18 days above 0.3 per cent. phenol, but a few cysts were still living at 1 and 0.5 per cent. in the

phenol agar and hay infusion after the same period of time. The *Cercomonas crassicauda* cysts by the eosin test showed that a few cysts were still living after seven days in 0.5 per cent. phenol hay infusion, but the cysts did not excyst when placed on normal agar; while as stated above the cysts of *Oikomonas termo* were killed with 0.3 per cent. phenol agar. Thus, amoebae cysts in some cases may be depressed by the phenol and after a long period may resume their activity when the medium has recovered from the influence of the phenol.

Since it was shown by Sewertzoff<sup>(10)</sup> that the dosage of antiseptics required for soil was much larger than in the case of cultures, it was decided to test the strengths of phenol on the protozoa in soil.

These experiments were done on the untreated soil from a tomato house, where the soil fauna was well known. The soil was taken to the laboratory, sieved, and divided into six portions of 100 gm. each. Each 100 gm. of soil was placed in large petri dishes, and sprayed with the following strengths of phenol, 0.15, 0.3, 0.6, 0.9, 1.8 per cent., an untreated soil acted as a control. All strengths below 1.8 per cent. showed the presence of protozoa though in the case of the 0.9 per cent. dilution very few amoebae and flagellates were found.

Further experiments were made with the same soil treated with 1.2, 2.4, 3.6 per cent. of phenol. No protozoa were found in the 3.6 per cent. dilution, and very few in the two lower dilutions.

Two plots of soil in a tomato house were treated with 0.25 per cent. and 0.15 per cent. carbolic acid, the numbers of protozoa were not so low as after steaming but the depressing effect, particularly on the amoebae, continued for over 55 days in 0.15 per cent. carbolic.

This work showed that the strengths of phenol used in commercial practice are too low to kill the protozoan cysts, though they cause an immediate disappearance of active forms from the soil for long periods of time.

Buddin<sup>(1)</sup>, who worked on the effect of partial sterilisation of soil by various antiseptics, found that  $M/10$ - $M$  killed off the protozoa,  $M/10$  being about equal to 1.2 per cent. which was used in these experiments. He found the same dose was necessary for cresol, which is one of the chief ingredients of the carbolic acid used in the above experiments.

#### THE INFLUENCE OF CARBON BISULPHIDE ON SOIL PROTOZOA.

Experiments on the influence of carbon bisulphide on soil protozoa were carried out on the same lines as in the previous work on the influence of phenol.

The following dilutions of carbon bisulphide were made up with agar and hay infusion, 1, 0.76, 0.5, 0.3, 0.14, 0.06, 0.02 per cent. and control normal agar.

### Experiments.

*Method.* Suspensions of cysts of *Hartmanella hyalina* were made in sterile tap water, 5 c.c. of which was inoculated into each of the following strengths (three plates for each), 1, 0.76, 0.5, 0.3, 0.14, 0.06, 0.02 per cent., and control.

A similar suspension of *Hartmanella hyalina* was inoculated into the same strengths of carbon bisulphide but hay infusion instead of agar was used, Table II illustrates the results both in the agar and hay infusion.

Table II.

*The influence of carbon bisulphide on Hartmanella hyalina cysts.*

Days	1 %		0.76 %		0.5 %		0.3 %	
	Agar	Hay	Agar	Hay	Agar	Hay	Agar	Hay
1	+	0	0	v f +	+	f +	+	+
3	+ a	0	+ a	0	+ a	f +	+	+
4	+ a	0	+ a	v f +	+ a	f +	+	+
9	+ a	0	+ a	0	+	0	+ a	+

Days	0.14 %		0.06 %		0.02 %		Control	
	Agar	Hay	Agar	Hay	Agar	Hay	Agar	Hay
1	+	+	+	+	+	+	+	+
3	+ a	+	+	+	+ a	+ a	+ a	+
4	+ a	+	+ a	+	+ a	+ a	a	+ a
9	+	+	+ a	+	+ a	+	a	+ a

+ = live cysts, a = actives, 0 = dead cysts or actives, v f = very few, f = few.

The experiments indicate that carbon bisulphide up to 1 per cent. in agar has no ill effect on cystic *Hartmanella hyalina*, which can excyst and continue to live in such agar; but carbon bisulphide in hay infusion has a decidedly depressing influence, a few cysts survived in 0.76 per cent. carbon bisulphide and lower dilutions for four days but no excysted actives were found above 0.02 per cent. after nine days.

Experiments were carried out on the influence of carbon bisulphide on active *Hartmanella hyalina* in cavity slips as above and kept in a damp chamber. The same range of dilutions were used as in the earlier experiments, but the first result of the treatment was to cause the *Hartmanella hyalina* to encyst, not to die. After three days, dead cysts

were found in the three higher dilutions, *i.e.* above 0.3 per cent., the same results continuing to the end of the experiment after fifteen days. The control had actives and cysts during the whole of the experiment.

The influence of carbon bisulphide on *Cercomonas crassicauda* cysts was studied, the dilutions 1, 0.76, 0.5, 0.3, 0.14, 0.06, 0.02 per cent. were used with hay infusion. It was found that at 0.5 per cent. and above the cysts were still found at the end of eleven days, while at 0.3 per cent. and below active *Cercomonas crassicauda* were found. In a similar experiment done on agar the results were similar.

When active *Cercomonas crassicauda* were treated with the same strengths of carbon bisulphide in hay infusion, in every case, except in the control, the *Cercomonas crassicauda* were killed off.

The experiments also show that the active forms of *Hartmanella hyalina* and *Cercomonas crassicauda* have a great difference in their tolerance of carbon bisulphide. Unless strong doses of carbon bisulphide are used for *Hartmanella hyalina*, the effect is to cause the actives to encyst and when the chemical has partially evaporated to excyst again, whereas *Cercomonas crassicauda* is killed.

#### THE EFFECT OF HEAT.

Soil heated to 60° C. was found by Russell and Golding<sup>(5, 6)</sup> to give a great increase in the number of bacteria but on the whole not such high numbers as when similar samples were treated with carbon bisulphide.

The same results, high numbers of bacteria, were again obtained by Russell and Petherbridge<sup>(9)</sup> in further experiments of heating the soil to 98° C. The effect was not found however when soil was heated to 50° C. or 55° C., the numbers in every case falling below those of the untreated soil. The soil in this case was kept at 55° C. for three hours. Heating soil to various temperatures was done by Russell and Hutchinson<sup>(7, 8)</sup> to find what temperature was required to kill off the detrimental factor, *i.e.* the protozoa in the soil. Various temperatures of 40° C. up to 65° C. were used, and the results showed that heating to 55° C. did not extinguish the factor but that in another soil heating at 50° C. for twelve hours temporarily extinguished the protozoa. 65° C. appeared to be ample, though the rise in numbers from 3 millions per gm. of soil to 60 millions in 210 days was not a very great increase.

*Methods.*

Cysts of *Naegleria gruberi* were placed in a test-tube containing sterile water, which was then placed in a container having water at the required temperature and kept there for a specified time. The viability of the cysts was then tested by the eosin method and by cultures.

*Experiments.*

The percentages killed as tested by eosin were: at 45° C. approximately 45 per cent., at 55° C. 71 per cent., at 65° C. 90 per cent., at 70° C. 98 per cent. or sometimes 100 per cent. It was thought that as the temperature of 70° C. gave such a high death rate, 75° C. would be a suitable temperature to test the amount of time necessary for the cysts under similar conditions to be exposed. Thus the experiment was repeated on the following day with cysts from the same culture, and with a mixed collection of cysts from several cultures heated to 75° C. for five and ten minutes.

The results with the eosin test gave 93 per cent. killed at five minutes and 100 per cent. killed at ten minutes for all cysts.

As a result of this experiment several others were done with sieved soil from the farm-yard manured plot from Barnfield. These showed that a temperature of 85° C. for fifteen minutes or longer is sufficient to kill off all the protozoa in 5 gm. of soil in a test-tube.

Another experiment with approximately 500 gm. of soil was carried out where light soil was sieved and divided into four portions, three of which were steamed for three different periods of time, seven, fifteen and thirty minutes, one being reserved as a control.

In the case of that heated for thirty minutes, no trace of any protozoa was found during the twenty-nine days that the soil was examined; in the soil heated for fifteen minutes a few flagellates and amoebae were occasionally found. The ciliates appear to be very susceptible to heat, as the actives and cysts were killed off after seven minutes in the steamer.

A further experiment, however, showed that thirty minutes at 99° C. is not absolutely lethal to all protozoa as both *Cercomonas crassicauda* and *Heteromita globosus* were present in small numbers in some further soil treated in that way.

These experiments show that a temperature of 99° C. is usually efficient in partially sterilising the soil if the heat is continued for thirty minutes. In one case when the soil was kept in the steamer for fifteen minutes after the steam had risen almost all the protozoa were killed,

a few flagellates being found on three samplings and amoebae twice out of twenty-nine samplings. Probably the physical conditions of the soil, such as the moisture content, will have an influence on the heat necessary for partial sterilisation.

Steaming experiments on tomato soil were done for two successive years. The temperature in the first year reached 100–105° C. at the edge of the plot, the steam being kept at the same pressure for one hour. The temperature at 9 in. after the steam was turned off was 98° C. The result of this treatment was to destroy almost all the bacteria and protozoa, though in one soil sample no protozoa were found. The protozoa numbers after twelve days were very depressed and were still low twenty-seven days after and continued depressed for some time. The steaming of the plots for the second year was not so drastic, the steam being continued for thirty minutes but the temperature did not rise above 60° C. This steaming reduced the numbers of the protozoa and bacteria so little that one of the plots was steamed a second time, the temperature rising on this occasion to 100° C. The protozoa, particularly in the twice steamed plot, showed a depression in numbers for 120 days after the steaming. As this lethal effect was too prolonged to be due only to the effect of steaming, a soil extract from the steamed soil was made, which proved lethal to active amoebae, though a similar extract from the untreated plot had no such effect.

#### SUMMARY.

(1) Experiments made to discover the death-point of protozoa by phenol, carbon bisulphide and heat showed that phenol has a greater lethal effect than carbon bisulphide.

(2) Heating the soil in a steamer for thirty minutes is usually sufficient to kill off the protozoa, and the treatment of glass-house soil by steam destroys the majority of the protozoa and has a depressing effect on their numbers for a long period.

#### REFERENCES.

- (1) BUDDEN, W. (1914). Partial Sterilisation of Soil by Antiseptics. *Journ. Agric. Sci.* vi, 417–451.
- (2) CUTLER, D. W. (1920). A Method for Estimating the number of Active Protozoa in the Soil. *Ibid.* x, Pt. II, pp. 135–143.
- (3) KESSEL, J. F. (1925). The Eosin criterion of the Vitality of Protozoan cysts as applied to cysts of *Hartmanella hyalina* treated with chlorine water. *Journ. Parasit.* II, 153.
- (4) MATTHEWS, A. (1924). Partial Sterilisation of Soil by Antiseptics. *Journ. Agric. Sci.* xiv, 1–51.

- (5) RUSSELL, E. J. (1913). The Complexity of the Micro-organic Population of the Soil. *Science*, xxxvii, No. 953, pp. 519-522.
- (6) RUSSELL, E. J. and GOLDING, J. (1912). Investigations on "Sickness" in Soil. (1) Sewage Sickness. *Journ. Agric. Sci.* v, Pt. i, pp. 27-47.
- (7) RUSSELL, E. J. and HUTCHINSON, H. B. (1909). The Effect of Partial Sterilisation of Soil on the production of Plant Food. *Ibid.* iii, Pt. ii, pp. 111-144.
- (8) ——— (1913). The Effect of Partial Sterilisation of Soil on the production of Plant Food. *Ibid.* v, Pt. ii, pp. 152-221.
- (9) RUSSELL, E. J. and PETHERBRIDGE, F. R. (1912). Investigations on "Sickness" in Soil. (II) Sickness in Glass House Soils. *Ibid.* v, Pt. i, pp. 86-111.
- (10) SEWERTZOFF, L. B. (1925). The Effect of some antiseptics on Soil Amoebae in Partially Sterilised Soils. *Centralb. f. Bakt.* II. Abt. Bd. Lxv, 278-291.
- (11) WAKSMAN, S. A. and STARKEY, R. L. (1923). Partial Sterilisation of Soil. Microbiological Activity and Soil Fertility. *Soil Sci.* xvi, 247-268.

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## CARBON DIOXIDE PRODUCTION IN SANDS AND SOILS IN THE PRESENCE AND ABSENCE OF AMOEBAE

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(With 2 Text-figures.)

If the functions of the micro-organisms in soil are to be fully understood a knowledge of their relation to the different chemical constituents of the soil is essential, so that the parts they play in breaking down or building up compounds can be evaluated. The present work was undertaken in an attempt to throw some light on the relation of bacteria and amoebae to carbon dioxide production and by this means to the organic matter in the soil. Such investigation is particularly interesting since it has been shown that amoebae are an important part of the active soil population and that their numbers are definitely related to those of the bacteria(4). Since the problem is one of great complexity only the simplest cases have hitherto been considered and the attempt has been confined to studying the action of one pure strain of bacteria, in the presence or absence of a pure line of amoebae, in sand cultures containing four different carbon sources, and in sterilised soils with four different manurial histories.

Previous work on carbon dioxide production from soil has largely been directed to attempting to correlate for field soils the carbon dioxide produced with their fertility, no effort being made to simplify the population; the literature of this subject is reviewed by Waksman and Starkey(15) and Waksman(14) and need not be considered here. All the work done has gone to show that carbon dioxide formation is conditioned by numerous factors, among which heat, moisture, source of carbon and type of organisms may be mentioned as the more important, and many investigators have concluded that it is safe to regard carbon dioxide production as a useful index of soil fertility, possibly of greater use in this connection than the actual numbers of bacteria present in the soil. Several observers (Stocklasa(12), Russell and Appleyard(10), Neller(7),

König and Hasenbäumer<sup>(6)</sup>, van Suchtelen<sup>(13)</sup>) found a definite correlation between carbon dioxide production and bacterial numbers. The amount of carbon dioxide produced by an unsterilised field soil is very variable; we have found that a well manured soil such as the dunged plot on Barnfield can give from 18 to 157 mgm. per kilo for the first 24 hours of an experiment, and an unmanured, 12 to 32. Waksman and Starkey recorded over 100 mgm. from one kilogramme of soil from a well manured, limed plot, and about 20 mgm. from an untreated soil.

A certain amount of work has also been done with pure cultures of bacteria inoculated into field soils; Westhues<sup>(16)</sup> found that sterile garden soil inoculated with three different species of bacteria gave amounts of carbon dioxide varying from 136 to 274 mgm. per 500 gm. in seven weeks.

Very little work has been done on the respiration of protozoa, one can therefore lay down no *a priori* expectation as to the amount of carbon dioxide that they will be responsible for producing in the soil. The little that is known is mostly derived from work on *Paramoecium*. Barratt<sup>(1)</sup> calculated that *Paramoecium* gave off from 1.3 to 5.3 per cent. of its body weight per day, and Pütter<sup>(9)</sup> calculated that this would represent 0.000035 mgm. per animal per day, but it must be remembered that Barratt's animals were in distilled water so that metabolism was at a low ebb and the figures quoted are therefore minimum and not average.

#### METHODS.

In the experiments under consideration both sands and soils were used as medium; the sand was silver sand which passed a 1 mm. sieve, it was washed with hydrochloric acid, and then with water until it was acid-free, and was then ignited. To the sand four different food sources were added in different experiments, after sterilisation, when the micro-organisms were inoculated. The four food solutions used were soil extract, mineral salt solution + 0.5 per cent. peptone, mineral salt solution + ammonium sulphate + 0.2 per cent. glucose (ratio C/N = 10), and mineral salt solution + sodium nitrate + 0.2 per cent. glucose (ratio C/N = 10).

The soils were taken from plots on Barnfield which have been under continuous cultivation since 1876, receiving respectively fourteen tons of farmyard manure, 550 lb. nitrate of soda and 400 lb. ammonium salts per acre per annum; the fourth plot received no manure. The soils all received the same treatment in the laboratory; they were air-dried, sieved through a 3 mm. sieve and the required amount was then autoclaved

in a two-litre flask for ten minutes at 15 lb. pressure. The sand was similarly autoclaved. Experience has shown that soil which is to be used as a medium for the growth of protozoa must not be subjected to a too drastic sterilisation, since toxic products are formed which inhibit growth(3). Pure cultures of "YB" bacteria(3) were employed, obtained from a single cell<sup>1</sup>, either with or without the addition of a pure mixed culture of *Hartmanella hyalina* with "YB," and every experiment consisted of parallel cultures of bacteria alone and bacteria + amoebae, so that the results are strictly comparable. The media were inoculated under aseptic conditions by spraying the cultures of bacteria or bacteria and amoebae on to the sand or soil from an Atlas spray, sufficient food solution in the case of sands or mineral salt solution in the case of soils being added to bring the medium to half the water-holding capacity. Bacteria and amoebae were counted daily by the dilution method in use in this laboratory(4).

The carbon dioxide evolved was estimated by the Pettenkoffer method using a baryta solution of about 0.1 per cent. in the tubes and titrating it against known hydrochloric acid of not more than *N*/5 strength. In every case the cultures were aerated by drawing carbon dioxide free air over them continuously by an aspirator. It has been shown by Potter and Snyder(8) that within wide limits the rate at which the air is drawn over the soil is immaterial; in these experiments the rate was between four and five litres in 24 hours. The carbon dioxide was estimated at least once in every 24 hours, that is, when the bacteria and amoebae were counted, but frequently the amount produced was so great as to necessitate more frequent titrations.

## RESULTS.

The growth of bacteria in an inoculated sand or soil follows closely the growth in an ordinary liquid culture; the numbers rise to a maximum within the first five days and then fall steadily with minor fluctuations; the presence of amoebae induces a greater tendency to fluctuate and lowers the bacterial numbers. Contrary to expectation, in the majority of cases the carbon dioxide production reaches its maximum a day before the bacterial numbers have reached theirs; then it drops sharply at first and more and more slowly until a steady level is maintained, at least for the duration of the experiments under discussion, which is a period of anything up to 15 days. Typical curves showing the carbon

<sup>1</sup> This pure strain of bacteria was prepared for us by Mr R. H. Stoughton using the "Dickinson Micro-Isolator."

dioxide production are given in Figs. 1 and 2. The amount of carbon dioxide produced in any given period is conditioned by a variety of factors as has been previously stated, but a rough idea of the amount

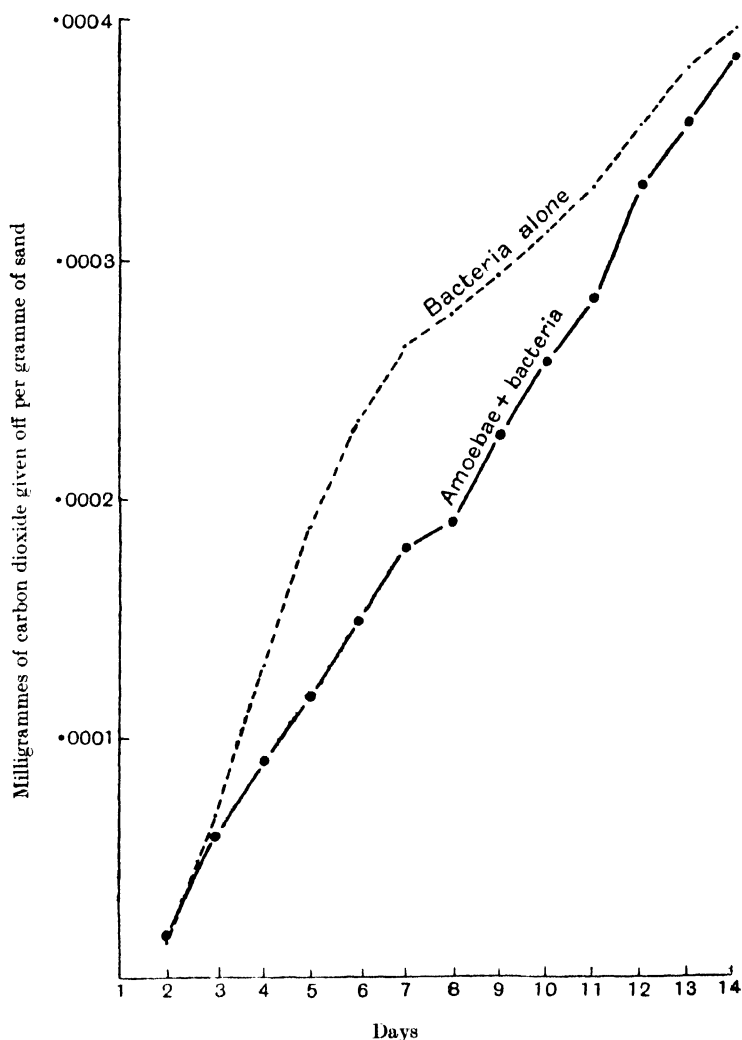


Fig. 1. Carbon dioxide production from bacteria and bacteria + amoebae in sand cultures with peptone.

of carbon dioxide which any given medium is capable of producing may be obtained from Table I, in which the amount of carbon dioxide given off during the first five days of an experiment is given.

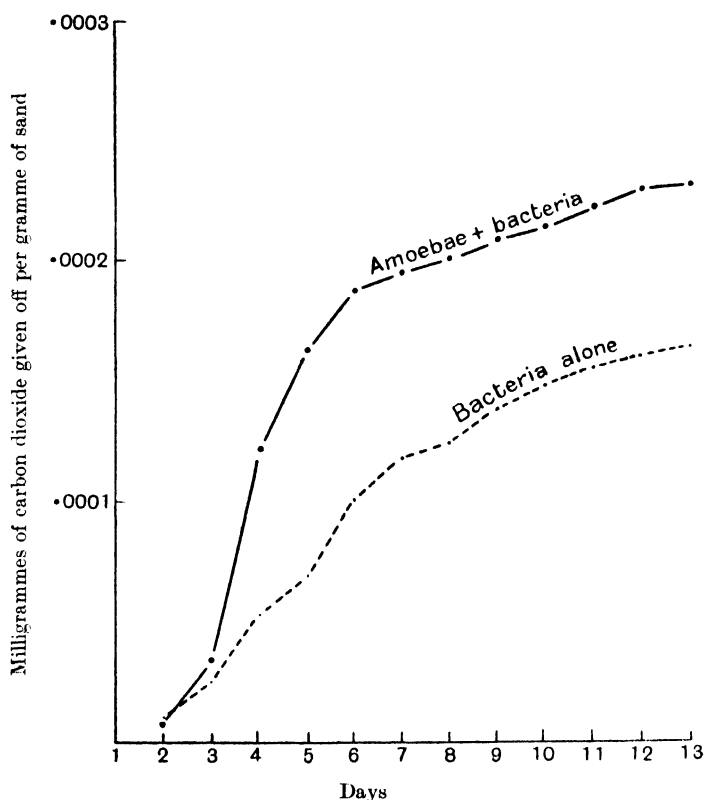


Fig. 2. Carbon dioxide production from bacteria and bacteria + amoebae in sand cultures with ammonium sulphate and glucose.

Table I.

*Average amount in gm. of carbon dioxide produced from 400 gm. of media in five days.*

Soils	Farmyard manured	Unmanured	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	NaNO <sub>3</sub>
	.1984	.0324	.0592	.0556
Sands	Peptone	Soil extract	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	NaNO <sub>3</sub>
	.1126	.0164	.0222	.0212

It is obviously of considerable interest to know what relation the numbers of bacteria bear to the formation of carbon dioxide; usually it has been assumed that there will be a definite relation between high numbers of bacteria and high carbon dioxide production and *vice versa*. The simplest method of testing this point since only two variables are involved is to arrange the data in contingency tables: in these an increase

in either variant is shown by a + sign and a decrease by a - sign; if both increase or decrease together it will be shown by a ++ or a --, if they vary inversely by a +- or -+. Where the two vary wholly independently there will be equality between the numbers of like and unlike signs, provided that there are a sufficient number of cases; a preponderance of like or unlike signs will show that the two variables are related to one another. To test the significance of any departure from equality a  $\chi^2$  may be worked out (5), and when a  $\chi^2$  has a greater value than 4 it may be assumed that there is a definite inter-relation between the two variables which is not due to chance. From Table II it appears

Table II.

*Contingency tables for carbon dioxide production and bacterial numbers in sand and soil cultures (CO<sub>2</sub> given first).*

Sands		++	+-	-+	--	$\chi^2$
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> or NaNO <sub>3</sub> + glucose	Bacteria alone	11	5	3	12	6.2
	With amoebae	10	4	6	13	5.1
Peptone	Bacteria alone	12	6	6	15	5.6
	With amoebae	10	8	10	12	0.4
Soil extract	Bacteria alone	15	7	4	11	6.1
	With amoebae	12	15	6	9	0.08
Soils		++	+-	-+	--	$\chi^2$
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> or NaNO <sub>3</sub>	Bacteria alone	9	20	9	17	—
	With amoebae	11	12	8	13	—
Farmyard manured	Bacteria alone	12	6	4	23	12.9
	With amoebae	7	6	3	13	3.9
Unmanured	Bacteria alone	3	8	5	7	—
	With amoebae	1	3	2	3	—

that where bacteria alone are present in sand cultures there is a very definite correlation between their numbers and the amount of carbon dioxide given off. The same is true of the soil receiving farmyard manure, but in the case of the unmanured soil and the soils receiving minerals there is no such correlation. There is no obvious reason for this anomalous result.

It is a matter of considerable interest to discover whether the bacterial capacity for producing carbon dioxide varies at all in the course of growth, and on different media, or whether it remains a constant. It appears from a study of different experiments that the carbon dioxide is very often produced in large quantities at the beginning of an experiment, even where in some cases the numbers of bacteria are still low. A contingency table was therefore made in which the two variables considered were the numbers of bacteria per gramme of medium,

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averaged for the beginning and end of each 24-hour period, and the amount of carbon dioxide produced per bacterium during that 24 hours, this giving a measure of the efficiency of the bacteria.

Table III.

*Contingency table for numbers of bacteria and efficiency in producing carbon dioxide (efficiency given first).*

Sands	++	+-	-+	--	$\chi^2$
Soil extract	10	13	14	5	4.8
Minerals + glucose	4	10	8	7	1.9
Peptone	5	10	11	4	4.8
All media	19	33	33	16	9.5
Soils	++	+-	-+	--	$\chi^2$
Farmyard manured	6	9	14	8	2.0
Unmanured	3	5	12	3	4.1
Minerals	6	12	27	11	7.1
All media	15	26	53	22	12.6

From Table III it is clear that in the majority of cases, both in sands and in soils, the bacteria are more efficient as producers of carbon dioxide when their numbers are not rising and less efficient as the numbers increase. That these results are not due to the fact that when low numbers of bacteria are present the culture is usually young and active and when high numbers are present it is staling, is shown in Table IV by considering the figures obtained when only the last five days of the culture's growth are considered.

Table IV.

Medium	++	+-	-+	--	$\chi^2$
Peptone	1	10	2	2	3.1
Soil extract	3	8	7	1	6.7
Minerals + glucose	0	7	3	0	—
All media	4	25	12	3	25.2

The obvious interpretation to place upon these results is that when the bacteria are reproducing, their energy is primarily devoted to building up fresh tissue, and that when reproduction is at a standstill, the energy is diverted and carbon dioxide is released in much larger quantities. In the case of young cultures, there is the anomaly that high efficiency for carbon dioxide production is correlated with active reproduction; apparently the bacteria of young cultures are able both to reproduce and to liberate carbon dioxide at the same time, whereas in the older cultures one or the other of the two processes predominates. These

contingency tables simply demonstrate that the efficiency varies according as to whether the numbers of bacteria in the cultures are increasing or decreasing, but they afford no information as to whether the efficiency is affected by the actual density of the population or as to whether it varies from medium to medium. If the efficiencies are grouped as in Table V, according to the number of bacteria present per gramme of

Table V.

*Bacterial efficiencies in gm. per 1000 million bacteria.*

		Numbers of bacteria in millions per gramme									
		No. of cases	0-200	No. of cases	200-400	No. of cases	400-600	No. of cases	600-800	No. of cases	Over 800
Soils											
Farmyard manured		2	·000245	2	·000140	7	·000126	3	·000098	33	·000039
Unmanured		12	·000149	9	·000053	1	·000055	2	·000049	2	·000049
NaNO <sub>3</sub>		24	·000365	10	·000058	5	·000062	1	·000029	—	—
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		16	·000190	7	·000091	—	—	—	—	1	·000041
Sands											
Peptone		4	·000092	11	·000080	9	·000042	5	·000105	5	·000063
Soil extract		28	·000259	8	·000068	5	·000058	—	—	7	·000017
NaNO <sub>3</sub>		14	·000558	1	·000112	—	—	—	—	—	—
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		19	·000790	—	—	—	—	—	—	—	—

Table VI.

*Amount of carbon dioxide in grammes given off from 400 gm. of different media by varying numbers of bacteria.*

		Numbers of bacteria in millions per gramme									
		No. of cases	0-200	No. of cases	200-400	No. of cases	400-600	No. of cases	600-800	No. of cases	Over 800
Soils											
Farmyard manured	Bacteria alone	—	—	—	—	—	—	1	·0228	32	·0216
	Bacteria and amoebae	—	—	—	—	1	·0256	1	·0404	31	·0240
Unmanured	Bacteria alone	11	·0072	9	·0056	1	·0100	1	·0128	2	·0068
	Bacteria and amoebae	11	·0068	7	·0068	4	·0104	—	—	2	·0440
NaNO <sub>3</sub>	Bacteria alone	13	·0072	8	·0052	4	·0072	1	·0092	—	—
	Bacteria and amoebae	15	·0068	8	·0072	—	—	—	—	2	·0076
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	Bacteria alone	16	·0104	4	·0112	—	—	—	—	1	·0160
	Bacteria and amoebae	17	·0100	7	·0112	1	·0072	—	—	1	·0152
Sands											
Peptone	Bacteria alone	4	·0056	11	·0084	9	·0088	5	·0272	5	·0252
	Bacteria and amoebae	14	·0076	9	·0136	6	·0160	2	·0112	2	·0300
Soil extract	Bacteria alone	27	·0052	8	·0072	5	·0116	—	—	3	·0104
	Bacteria and amoebae	20	·0044	11	·0172	3	·0080	1	·0068	8	·0064
NaNO <sub>3</sub>	Bacteria alone	20	·0040	1	·0096	—	—	—	—	—	—
	Bacteria and amoebae	20	·0088	—	—	—	—	—	—	—	—
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	Bacteria alone	20	·0036	—	—	—	—	—	—	—	—
	Bacteria and amoebae	19	·0060	—	—	—	—	—	—	—	—

medium, it is seen that the fewer bacteria there are present the more efficient each one becomes. Information as to the influence of the medium on the efficiency of the bacteria can be obtained by considering the actual amount of carbon dioxide given off by a constant volume of medium with the same numbers of bacteria. From Table VI, though the distribution is irregular, it would appear that although an increased population gives off an increased amount of carbon dioxide on any one medium, yet the bacteria produce roughly the same amount of carbon dioxide, irrespective of the medium. The different media are unable to support the same sized populations, since in the soils the unmanured and those with minerals rarely have a density of over 400 millions per gm., while the farmyard manured are almost entirely above 800 millions. This is also shown, though in a lesser degree, by the sand experiments, where peptone supports a larger population than the others.

#### *Effect of amoebae.*

In both sands and soils the presence of amoebae has the usual result of lowering the numbers of bacteria, but the effect upon carbon dioxide production is not so simple. In Table II the correlation between carbon dioxide production and bacterial numbers, when amoebae are present, is not so sharp as when bacterial cultures alone are considered; thus on soils there is no correlation in the unmanured and minerals, and in sand the same obtains for peptone and soil extract, but the ammonium sulphate and nitrate of soda sand cultures give a  $\chi^2$  over 4 and the farmyard manured soils one of 3.9. Such divergent results are only to be expected when it is remembered that the amount of carbon dioxide given off by amoebic respiration would often be sufficiently great to mask the direct effect from carbohydrate decomposition; and that this latter is conditioned by the continual fluctuations between the numbers of bacteria and amoebae. When the action of the amoebae on the amount of carbon dioxide produced is considered it is found that, in the case of sand cultures, amoebae decrease the amount of carbon dioxide given off when peptone is used, but increase the amount in the case of soil extract and of the mineral solutions with glucose (Figs. 1 and 2). When comparing each 24-hour period for the cultures, with or without amoebae, in soil extract, on 76 per cent. of the occasions more carbon dioxide was produced when amoebae were there; in the case of mineral salts and glucose there were 66 per cent. of times when the carbon dioxide was higher with amoebae, but in the case of peptone on 67 per cent. of the occasions the carbon dioxide was less in the presence of amoebae.

If the exceptions are examined the numbers are found to be even more significantly different than appears from the figures quoted. For instance in the soil extract cultures there are nine occasions when less carbon dioxide is produced where amoebae are present, but on five of these the bacteria are lower than is the case in the cultures of bacteria alone and the numbers of amoebae are negligible, and the same holds good for six out of the fourteen exceptions for the cultures with mineral salts and glucose. In the peptone experiments there are twelve cases where the amoebic cultures give more carbon dioxide than do the purely bacterial ones. In five of these the bacterial numbers are not significantly different in the two series of cultures, but the numbers of amoebae are very high, and the amoebic respiration might well bring up the output of carbon dioxide; in one case the bacteria were distinctly higher in the amoebic cultures and in two others the bacterial numbers were higher and the amoebae were also present in large numbers. Excluding therefore those which are explicable as shown above, the percentages of exceptions are reduced to 11, 20 and 10 per cent. in the case of soil extract, minerals and peptone respectively.

In soils the results are not so clear cut; in the case of the farmyard manured soil where the expectation is that less carbon dioxide would be produced in the presence of amoebae, as is the case with peptone in sand, in actual fact this only occurred on 47 per cent. of the occasions, and out of the seventeen exceptions only seven could be explained as being due to the numbers of bacteria being higher in the series with amoebae. The unmanured soil fell more into line with the sand treated with soil extract, in that the carbon dioxide produced was greater in 64 per cent. of the cases where amoebae were present, but of the ten exceptions only one could be due to the small numbers of both bacteria and amoebae in the amoebic cultures. The soils from the ammonium sulphate and nitrate of soda plots were again unsatisfactory, since only 50 per cent. of the cases gave higher carbon dioxide in the presence of amoebae, and of the fourteen exceptions only three could be accounted for by the low numbers of bacteria and amoebae in these cultures.

In view of the complex nature of soil compared with the simple inoculated sands it would be expected that the results would not be so clear cut; further work is therefore contemplated on soils together with experiments testing the amount of carbon dioxide which can be ascribed to amoebic and bacterial respiration as distinct from that produced by carbohydrate decomposition.

## SUMMARY.

Experiments are described on carbon dioxide production from soil and sand cultures containing a species of bacterium with and without amoebae. The following results were obtained:

1. Carbon dioxide production and bacterial numbers are correlated provided that amoebae are not present, or are present in very small numbers.

2. The bacteria are more efficient as producers of carbon dioxide when their numbers are not rising and less efficient when their numbers are increasing. This does not hold for young cultures. Also each bacterium becomes less efficient as the density of the population increases.

3. The amoebae cause a decrease in carbon dioxide production in sands containing peptone, but an increase in sands containing mineral salt solution with glucose or soil extract

## REFERENCES.

- (1) BARRATT, J. O. W. (1905). Die Kohlensäureproduktion von *Paramoecium aurelia*. *Zeitschr. f. Allgem. Physiol.* v, 66-72.
- (2) CUTLER, D. W. and BAL, D. V. (1926). Influence of Protozoa on the process of nitrogen fixation by *Azotobacter chroococcum*. *Ann. App. Biol.* xiii, 516-534.
- (3) CUTLER, D. W. and CRUMP, L. M. (1927). The Qualitative and Quantitative effects of food on the growth of a soil amoeba (*Hartmannella hyalina*). *Brit. Journ. Exper. Biol.* v, 155-165.
- (4) CUTLER, D. W., CRUMP, L. M. and SANDON, H. (1922). A Quantitative investigation of the Bacterial and Protozoan Population of the Soil. *Phil. Trans. Roy. Soc. B.* 211, 317-350.
- (5) FISHER, R. A. (1928). *Statistical Methods for Research Workers*. Edinburgh.
- (6) KÖNIG, J. and HASENBÄUMER, J. (1920). Die Bedeutung neuer Bodenforschungen für die Landwirtschaft. *Landw. Jahrb.* lv, 185-252.
- (7) NELLER, J. R. (1920). The oxidising power of soil from limed and unlimed plots and its relation to other factors. *Soil Sci.* x, 29-39.
- (8) POTTER, R. S. and SNYDER, R. S. (1916). Carbon dioxide production in soils, and carbon and nitrogen changes in soils variously treated. *Iowa Agric. Stat. Res. Bull.* 39, 255-309.
- (9) PÜTTER, A. (1905). Die Atmung der Protozoen. *Zeitschr. f. Allg. Physiol.* v, 566-612.
- (10) RUSSELL, E. J. and APPLEYARD, A. (1915). The Composition of the soil atmosphere. *Journ. Agric. Sci.* vii, 1-48.
- (11) STOKLASA, J. and ERNEST, A. (1905). Ueber den Ursprung der Menge und die Bedeutung des Kohlendioxyds im Boden. *Centralbl. Bakt.* ii, 14, 723-736.
- (12) STOKLASA, J. (1912). *Abderhald. Handb. biochem. Arbeitsmeth.* v, pt 2, 1925.
- (13) VAN SUCHTELEN, F. H. H. (1910). Ueber die Messung der Lebenstätigkeit der aerobiotischen Bakterien im Boden durch die Kohlensäureproduktion. *Centralbl. Bakt.* ii, 26, 45-89.
- (14) WAKSMAN, S. A. (1927). *Principles of Soil Microbiology*. London.
- (15) WAKSMAN, S. A. and STARKEY, R. L. (1924). Microbiological analysis of Soils as an index of soil fertility. *Soil Sci.* xvii, 141-161.
- (16) WESTHUES, J. (1905). Die Kohlensäurebildung im Boden. *Inaug. Dissert. Münster i. W.*

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## THE GROWTH OF FUNGI IN SOIL<sup>1</sup>

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(With 1 Text-figure.)

### I. INTRODUCTION.

It has long been recognised that the fungi are a normal constituent of the soil flora, but the condition in which they are present in the soil has been, and still is, a debated point. Waksman<sup>(12)</sup> devised a method which enabled him to state that some fungal forms, at least, exist in the soil in an active mycelial condition. He placed lumps of soil, procured under sterile conditions, on to plates of Czapek's agar. After an incubation period of 24 hours hyphae were found to radiate from the edge of the soil sample into the medium. The short incubation period excluded the possibility of spore origin for the mass of mycelium formed, for similar plates inoculated at the same time with spores alone, showed only minute colonies just discernible to the naked eye.

Brown<sup>(4)</sup> repeated these experiments and confirmed the results. Conn<sup>(6)</sup>—who advocates a direct microscopic examination of the soil for arriving at a quantitative estimation of the numbers of soil organisms—reported the almost entire absence of mould hyphae from soil smears examined by him and stained as directed with Rose Bengal in carbolic acid—a method primarily designed for the detection of bacteria in soil in this direct way. Later (1922) he modified the technique and substituted wet mounts instead of dry and used as a stain methylene blue, with the result that he could demonstrate fungus filaments in practically every soil he examined, but still they proved to be far from abundant.

Winogradsky<sup>(17)</sup> employs the direct microscopic method in his study of the soil and favours the view that the fungal constituents of the soil are present as spores, which become active, and therefore converted into the mycelial phase on the addition of an organic substance, *e.g.* cellulose, to the soil.

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Parallel with the above issue and to be considered alongside with it, may be ranked the attempts made to determine the number of fungi in any soil (Waksman<sup>(13, 14)</sup> and Brierley<sup>(2)</sup>). The method most usually employed has been a dilution method, whereby a fraction of a soil suspension after suitable dilution is plated out in a series of petri dishes which are later poured with a cooled nutrient medium, incubated for 5-9 days and the number of fungal colonies appearing on each plate then counted. From this the number present in the original sample are calculated. The factors involved in this quantitative technique have been more recently studied by Brierley, Jewson and Brierley<sup>(3)</sup> of the Rothamsted Experimental Station and many of their methods have been adopted in the present investigation.

Conn<sup>(6)</sup> has pointed out that a large plate count may simply be due to the fact that an organism may have sporulated and may not necessarily indicate that fungi are playing any outstanding rôle in that particular soil. The following experiments are of interest in this connection.

## II. GROWTH OF PURE CULTURES OF FUNGI IN STERILISED SOIL.

Soil which had been sterilised by autoclaving was inoculated with equal numbers of spores of each of four different fungi, viz. *Alternaria humicola* Oud., *Penicillium lilacinum* Thom., *Trichoderma Koningi* Oud. and *Verticillium (Acrostalagmus) cinnabarinus* Corda. from cultures previously isolated by Brierley from Rothamsted soils. The number of spores per 1 c.c. of inoculum was determined by means of a Bürker haemocytometer. One c.c. of a suspension containing 3040 spores (760 of each genus) was added to 300 gm. of sterile soil, i.e. 10 spores for every gram of soil. This was kept at 9° C. together with an uninoculated control flask. At the same time triplicate plates of three different media (soil agar, Coons' agar and Conn's glycerine sodium-asparaginate agar) were inoculated in the centre with spores of the above fungi and incubated alongside the flasks. A temperature of 9° C. was chosen as convenient, for it delayed the sporing phase of some of the forms (see Fig. 1) and thus enabled one to compare the relative number of colonies of sporing and non-sporing forms which developed on the plates. The rate of growth of these forms in pure culture at this temperature was measured in terms of the increase in diameter of the colonies from day to day.

One week after the flasks had been inoculated, 10 gm. of soil were removed under sterile conditions and plates poured by the dilution method. Eight plates each for a range of dilutions from 1/80 to 1/20,480 were poured with the result set out in Table I.

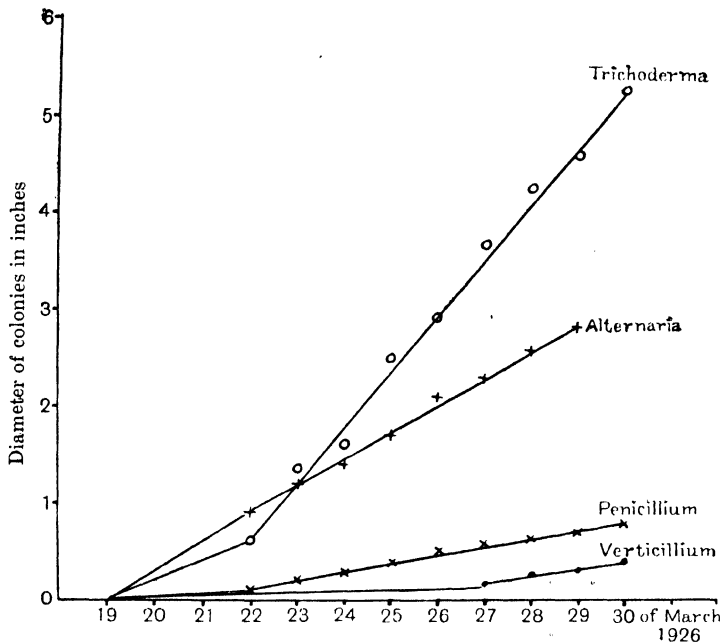


Fig. 1. The diameter represents the average of the growth on three media, viz. soil agar, Coon's agar and Conn's agar. Plates incubated at 9° C.

Table I.

*The number of colonies obtained over a wide range of dilutions from a soil sample one week after inoculation with a mixture of spores from four different kinds of fungi.*

Dilution	Average number of colonies per plate
1 in 80	40.37 ± 1.7
1 in 160	20.87 ± 1.7
1 in 320	9.43 ± 0.9
1 in 640	4.62 ± 0.8
1 in 1,280	3.12 ± 0.4
1 in 2,560	1.62 ± 0.4
1 in 5,120	0.50 ± 0.15
1 in 10,240	0.25 ± 0.13
1 in 20,480	0.12 ± 0.04

An analysis of the population showed that at the end of seven days the only form appearing on the plates was *Alternaria humicola*.

Reference to Fig. 1 shows that at this date both *Alternaria humicola* and *Trichoderma Koningi* in pure culture had approximately equal colony

Table II.

*An analysis of the population appearing on the plates one week after the inoculation of the soil sample.*

Plates ...	1				2				3				4			
Dilution	A	P	V	T	A	P	V	T	A	P	V	T	A	P	V	T
1 in 80	45	.	.	.	36	.	.	.	42	.	.	.	39	.	.	.
1 in 160	16	.	.	.	19	.	.	.	16	.	.	.	28	.	.	.
1 in 320	10	.	.	.	11	.	.	.	6	.	.	.	13	.	.	.
1 in 640	4	.	.	.	3	.	.	.	6	.	.	.	2	.	.	.
1 in 1,280	3	.	.	.	3	.	.	.	3	.	.	.	5	.	.	.
1 in 2,560	.	.	.	.	2	.	.	.	2	.	.	.	1	.	.	.
1 in 5,120	1	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.
1 in 10,240	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.
1 in 20,480	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.

Plates ...	5				6				7				8			
Dilution	A	P	V	T	A	P	V	T	A	P	V	T	A	P	V	T
1 in 80	44	.	.	.	47	.	.	.	34	.	.	.	36	.	.	.
1 in 160	24	.	.	.	17	.	.	.	23	.	.	.	24	.	.	.
1 in 320	7	.	.	.	Spoilt				9	.	.	.	10	.	.	.
1 in 640	8	.	.	.	4	.	.	.	8	.	.	.	2	.	.	.
1 in 1,280	3	.	.	.	5	.	.	.	1	.	.	.	2	.	.	.
1 in 2,560	.	.	.	.	3	.	.	.	2	.	.	.	3	.	.	.
1 in 5,120	1	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.
1 in 10,240	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1 in 20,480	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

*A* = *Alternaria humicola*, *P* = *Penicillium lilacinum*, *V* = *Verticillium cinnabarinus*, *T* = *Trichoderma Koningi*.

Table III.

*The number of colonies per plate eleven days after the inoculation of the soil sample.*

Dilution	Average number of colonies per plate
1 in 80	Too crowded for accurate counts
1 in 160	
1 in 320	
1 in 640	132.75 ± 4.9
1 in 1,280	70.87 ± 0.88
1 in 2,560	38.25 ± 2.1
1 in 5,120	19.37 ± 1.15
1 in 10,240	12.5 ± 1.3
1 in 20,480	5.62 ± 0.78

diameters. *Alternaria*, however, was sporing whereas *Trichoderma* was entirely in the vegetative condition, and the mycelial development was apparently not sufficiently great to produce colonies with the amount of fragmentation induced and the dilutions used.

Eleven days after inoculation a further 10 gm. of soil were removed after thoroughly shaking the flasks, diluted through the same range and plated out as before (Tables III and IV).

Table IV.

*Analysis of the population after eleven days of incubation  
of the soil sample.*

Plates ...		1				2				3				4			
Dilution		A	P	V	T	A	P	V	T	A	P	V	T	A	P	V	T
1 in	80	Rest	15	.	.	Rest	4	.	.	Rest	7	.	.	Rest	2	.	.
1 in	160	Rest	2	1	.	All	.	.	.	Rest	3	.	.	Rest	.	2	.
1 in	320	Rest	3	.	.	Rest	3	.	.	Rest	4	.	.	All	.	.	.
1 in	640	147	1	.	.	143	3	.	.	129	.	.	.	135	2	.	.
1 in	1,280	69	1	.	.	68	.	.	.	70	1	.	.	71	3	.	.
1 in	2,560	30	1	.	.	38	.	.	.	45	1	.	.	46	.	.	.
1 in	5,120	19	.	.	.	21	.	.	.	26	.	.	.	16	.	.	.
1 in	10,240	19	.	.	.	15	.	.	.	9	.	.	.	14	1	.	.
1 in	20,480	5	.	.	.	2	.	.	.	5	.	.	.	4	.	.	.

Plates ...		5				6				7				8			
Dilution		A	P	V	T	A	P	V	T	A	P	V	T	A	P	V	T
1 in	80	Rest	11	.	.	Rest	3	.	.	Rest	10	1	.	Rest	8	.	.
1 in	160	Rest	5	.	.	Rest	4	1	.	Rest	5	.	.	Rest	7	.	.
1 in	320	All	.	.	.	Rest	2	.	.	Rest	3	1	.	Rest	2	.	.
1 in	640	119	1	.	.	109	1	.	.	125	1	.	.	144	2	.	.
1 in	1,280	70	1	.	.	74	1	.	.	69	1	.	.	67	1	.	.
1 in	2,560	32	.	.	.	42	.	.	.	32	.	.	.	39	.	.	.
1 in	5,120	16	1	.	.	17	.	.	.	18	.	.	.	21	.	.	.
1 in	10,240	9	1	.	.	12	.	.	.	7	.	.	.	13	.	.	.
1 in	20,480	8	.	.	.	7	.	.	.	5	.	.	.	9	.	.	.

The analysis again shows that the population of the plates was almost entirely *Alternaria humicola*, although the mycelial development of *Trichoderma Koningi* measured in terms of its colony diameter in pure culture is far in excess of that of *Alternaria*. In the lower dilutions a few colonies of *Penicillium lilacinum* appeared. Sporing in this case evidently occurred at about this time but was not evident to the eye in the pure cultures until the 13th day.

Twenty-seven days after inoculation 10 gm. were again removed and plated as before (Tables V and VI).

Table V.

*Number of colonies per plate twenty-seven days after the inoculation of the soil sample.*

Dilution	Average number of colonies per plate
1 in 1,280 } 1 in 2,560 }	Plates too crowded
1 in 5,120	103.87 $\pm$ 2
1 in 10,240	47.87 $\pm$ 0.9
1 in 20,480	29.62 $\pm$ 2.8

Table VI.

*Analysis of the population twenty-seven days after inoculation.*

Plates...	1				2				3				4			
Dilution	A	P	V	T	A	P	V	T	A	P	V	T	A	P	V	T
1 in 1,280 Rest	124	4	2		Rest	111	5	2	Rest	134	2	2	Rest	109	1	2
1 in 2,560 Rest	79	6	1		Rest	63	6	2	Rest	98	9	2	Rest	84	7	3
1 in 5,120	50	43	4	2	54	44	6	2	52	40	6	2	50	50	4	.
1 in 10,240	21	27	3	1	18	28	2	.	25	20	3	2	20	25	2	1
1 in 20,480	16	17	3	.	10	10	4	.	14	17	1	.	11	11	2	.

Plates...	5				6				7				8			
Dilution	A	P	V	T	A	P	V	T	A	P	V	T	A	P	V	T
1 in 1,280 Rest	153	4	2		Rest	130	6	1	Rest	135	4	3	Rest	124	5	2
1 in 2,560 Rest	71	10	1		Rest	92	7	1	Rest	90	7	.	Rest	70	8	2
1 in 5,120	51	39	6	2	53	45	4	1	53	56	5	2	52	49	9	.
1 in 10,240	19	25	1	.	23	25	.	.	20	22	1	.	29	18	2	.
1 in 20,480	12	13	1	1	16	12	.	.	13	23	2	.	13	14	1	.

At the end of twenty-seven days the numbers of *Penicillium* and *Alternaria* colonies were approximately equal to one another. This is due to the large number of *Penicillium* spores as opposed to the smaller number of large spores in *Alternaria* but reference to Fig. 1 shows the wide variance in the amount of mycelial development in the two cases.

These results show very clearly that the number of fungi occurring in soil, calculated by the dilution-plating method, can give no idea of the relative abundance or extent of the forms *actively* growing at any time in this substratum. Conn<sup>(6)</sup> attributed the paucity of fungal mycelium in soil as revealed by his technique to the fact that "fungus growth is not sufficiently abundant in that particular soil to show under the microscope." He further states that there is no real inconsistency between the high counts of fungi reported by Brierley<sup>(2)</sup>, Waksman<sup>(14)</sup> and others, and his own small estimates, "for a colony of *Aspergillus*

growing on an agar plate bore spores enough to give a plate count of 300,000 per gm. provided they were distributed evenly throughout a kilo of soil and every spore was capable of growth, yet such a small amount of mycelium would be added to the soil that only one fragment of mould hyphae would be found in every 3000 microscopic fields."

The only conclusion one can draw from these statements is that Conn considers that the fungi are present in the soil largely in the spore state, and, so, high plate counts and feeble development of mycelium in soil, if this assumption is correct, are in no way contradictory.

### III. EFFECT OF DRYING UNDER CONTROLLED CONDITIONS ON THE FUNGAL POPULATION OF THE SOIL.

The questions then arise: "What is the condition of the fungi in the soil?" "Do spores predominate over vegetative mycelia?" and "What interpretation are we to place on plate count results?"

Waksman, as already pointed out, has demonstrated the fact that some active mycelium does occur in the soil, but apart from this there has been no attempt to try and measure the extent of this active phase or, indeed, to separate the active and spore members of the soil. In order to reach some conclusion in this matter the following experiments were performed.

A sample of soil was collected under sterile conditions and from it two representative samples of 10 gm. each were weighed out.

One of these 10 gm. samples was immediately placed in 100 c.c. of sterile water and vigorously shaken for half an hour. Fifty c.c. of this suspension was transferred by sterile pipettes to 50 c.c. of sterile water and so on until the desired dilution was reached. Sixteen 1 c.c. portions of the final dilution were then placed in petri dishes and the plates poured with Conn's glycerine sodium-asparaginate agar of a pH 4.7. The plates were incubated at room temperature and counts made of the colonies which developed.

The other sample was transferred to a sterile petri dish and placed in a desiccator over calcium chloride to dry. In order to hasten drying a vacuum desiccator was chosen and a cotton-wool plug was inserted between the pump and tap of the desiccator when the latter was disconnected from the pump. Air, before entering, had to filter through the plug and so the entrance of air-borne spores to the soil by the inrush of air was prevented.

The sample was left till dry; the usual time was three days, but drying was evident in a much shorter time. It was then removed from the

Table VII.

*A comparison of the number of colonies obtained from an untreated soil sample and the number obtained from a similar sample that had been dried in a desiccator before plating.*

Sample A. Dilution 1 in 20,480. Plates incubated for 10 days (counts made on the 3rd, 5th, 7th and 10th day).

Plates	Untreated soil		Treated soil	
	Total population	Fungal population	Total population	Fungal population
1	81	11	3	0
2	77	7	3	1
3	64	8	3	0
4	118	16	0	0
5	57	13	1	0
6	67	14	3	2
7	61	6	0	0
8	96	10	2	0
9	61	11	3	0
10	107	8	0	0
11	55	7	6	2
12	98	11	2	2
13	100	16	1	0
14	78	11	3	3
15	63	10	2	0
			3	0
Average	78.8 $\pm$ 5.2	10.6 $\pm$ 0.8	2.1 $\pm$ 0.16	0.62 $\pm$ 0.25

Table VIII.

*Number of colonies obtained from a similar sample (see Table VII) which had been stored in the laboratory while desiccator sample was drying.*

Plates	Total population	Fungal population
1	43	10
2	62	11
3	53	7
4	33	8
5	68	8
6	23	7
7	35	7
8	25	10
9	116	9
10	55	9
11	60	8
12	52	8
13	196	7
14	76	10
15	33	7
Average	62 $\pm$ 11.3	8.4 $\pm$ 0.3

desiccator and plated in identically the same way as the untreated sample, the same batch of medium being used in both cases. The results of the two platings were then compared (see Table VII).

As a check against this result a 10 gm. portion of the same soil sample was placed in a sterile container which was closed with a cotton-wool plug and kept in the laboratory for the same length of time as the sample was kept in the desiccator. This was plated out in the same way and at the same time as the latter. The results are shown in Table VIII.

As there is no significant difference between these results and those from the untreated sample, any biological changes taking place in the soil during the storage-period of three days does not account for the decrease in numbers obtained on plating out the treated sample.

The results for similar samples are given in the following tables.

Table IX.

*Averages obtained from further samples subjected to the same treatment.*  
*Dilution 1/20,480.*

Sample	Period of incubation (days)	No. of plates	Untreated soil		Treated soil	
			Total population	Fungal population	Total population	Fungal population
B	8	16	33.7 $\pm$ 3.35	11.56 $\pm$ 0.84	4.43 $\pm$ 0.77	1 $\pm$ 0.18
C	12	16	163.7 $\pm$ 9.17	8.8 $\pm$ 0.74	84.5 $\pm$ 4.01	1.06 $\pm$ 0.30
D	9	8	142.5 $\pm$ 3.7	25.2 $\pm$ 1.8	126.75 $\pm$ 4.2	3 $\pm$ 0.71

As the fungal colonies were practically eliminated from the plates by this treatment when using a dilution of 1 in 20,480, *lower dilutions* were next used and parallel results were obtained, as shown in Table X.

Table X.

Sample	Period of incubation (days)	No. of plates	Fungal colonies*		Dilution
			Untreated soil	Treated soil	
E	7	16	199 $\pm$ 4.37	13.7 $\pm$ 0.8	1 in 320
F	7	16	326.25 $\pm$ 18.15	5.56 $\pm$ 0.52	1 in 640

\* These plates were examined microscopically and the fungal colonies counted in this way, for owing to the large number developing on the plates many did not reach macroscopic proportions.

In order to see if the reduced pressure was in any way responsible for the result in addition to the drying factor, a sample of soil was placed in the desiccator and allowed to dry over calcium chloride at ordinary air pressure. When dry it was plated out as before. The results are given in Table XI.

Table XI.

*Number of colonies per plate from soil dried at air pressure.*

Plates	Untreated		Treated (dried at air pressure)	
	Total population	Fungal population	Total population	Fungal population
1	162	9	74	0
2	180	13	55	4
3	135	11	88	0
4	191	7	90	0
5	115	4	62	1
6	172	10	68	1
7	184	7	76	1
8	166	8	110	2
9	198	9	86	1
10	137	10	69	2
11	144	9	116	0
12	170	8	107	4
13	155	11	72	1
14	186	6	93	1
15	193	14	68	2
16	206	9	77	5
Average	168.37 $\pm$ 6.07	9 $\pm$ 1.57	81.93 $\pm$ 4.41	1.56 $\pm$ 0.37

Experiments were then made to determine the length of time it was necessary to leave the soil sample in the desiccator to obtain a definite depression in the number of fungal colonies developing on the plates (Table XII).

Table XII.

*Effect of varying periods of time in desiccator on number of colonies per plate.*

Time in desiccator (hours)	Period of incubation (days)	No. of plates	Total population	Fungal population
0 (control)	7	16	196.5 $\pm$ 3.75	8.25 $\pm$ 0.465
4	7	16	174.43 $\pm$ 3.35	8 $\pm$ 0.24
18	7	16	121.8 $\pm$ 8.9	1.56 $\pm$ 0.29

Summarising these results, it is evident that when soil is dried in such a way as to exclude air contamination and then plated out by the dilution method the number of fungal colonies per plate is markedly decreased. In fact the whole population tends to show this depression; in some soils this is much more marked than in others.

Allison(1), when working on the biological changes in soil during storage, made a few determinations on the numbers of organisms in

air-dried soil as against the numbers present in a moist sample of the same soil. He found that air-drying caused a decided decrease in bacterial numbers in most cases, but the extent of the decrease seemed to depend largely on the kind of soil, a conclusion which is supported by the above figures. Too much reliance however should not be placed on the bacterial counts, for the acid medium used suppresses the development of many of the soil forms. He did not obtain any marked decrease for fungi, but this is not surprising as the soil was spread out to dry "in the open air of the dark incubating room."

These facts seem to indicate that the fungi are present in the soil almost exclusively in the *active mycelial condition* and when the soil is dried the hyphae are killed and therefore the low count results. That there is no induced formation of spores as a result of the subjection of the sample to drying is also evident from the low figures obtained. It may be argued that fungi inoculated into sterile soil not only grow luxuriantly but *spore* abundantly. However, soil which has been autoclaved is radically altered from a physical, a chemical and a biological standpoint. In its enriched condition, which is accompanied by a complete suppression of all biological competition, it serves simply as a good nutrient medium and consequently fungi inoculated into it run riot and carry out their normal life-cycle.

In order to obtain further evidence to support the contention, that the mycelial phase is all-important in the soil, a suspension of fragmented pieces of mycelia of *Trichoderma Koningi*, which had been grown in Richard's solution, was thoroughly shaken up with sand and also with sterilised soil. The resulting samples were placed in sterile petri dishes, transferred to the desiccator, the lids removed and left to dry out over calcium chloride and were finally plated out at a dilution of 1 in 640. A similar suspension was plated out directly at the same dilution. The number of colonies which developed per plate were compared in the two cases (Table XIII).

Table XIII.

Nos. of colonies	
Plated immediately,	Plated after drying
20	1
18	0
21	1
25	2
16	0

Similarly, a suspension was made of the spores of four different fungi (*Penicillium lilacinum*, *Verticillium cinnabarinus*, *Trichoderma Koningi* and a green *Penicillium*) and 2 c.c. of this suspension was thoroughly mixed with 10 gm. of sterilised soil and placed in the desiccator to dry, after which it was plated out at a dilution of 1 in 2560. Two c.c. of the same suspension was then shaken in another 10 gm. of soil; this was plated out directly at the same dilution and the number of colonies per plate in each case were again compared (Table XIV).

Table XIV.

Nos. of colonies	
Plated immediately	Plated after drying
34	25
30	28
26	28
29	30
36	39

These results show clearly that drying of the soil samples would not prevent the subsequent germination of the fungal spores if present in the soil when plated out on a nutrient medium, whereas all the mycelial hyphae can be eliminated by this method and a comparison of the counts both before and after drying gives a measure of the activity of fungi in any particular soil. High plate counts, therefore, obtained by the dilution method suggest large mycelial development.

A further experiment was devised to test this point. Samples, each of 10 gm. of soil, were placed in a series of petri dishes and autoclaved at 20 lb. pressure for three periods of 20 mins. each. To each was then added 1 c.c. of a suspension of spores of three different species of soil fungi, viz. *Penicillium* sp. *Verticillium cinnabarinus* and *Macrosporium* sp. One sample was plated immediately on to Conn's glycerine sodium-asparaginate agar at a dilution of 1 in 20,480 (series A), a corresponding sample was placed immediately in a vacuum desiccator over calcium chloride and after two days similarly plated (series A<sub>1</sub>). The remainder were incubated, one pair of samples for *three days*, the other for *six days* at 25° C. At the end of the third day, one was again plated at the same dilution without drying; a corresponding sample was placed in the desiccator for two days and then plated (series B and B<sub>1</sub>). At the end of the sixth day one of the remaining samples was plated directly at a dilution of 1 in 655,360—this dilution was necessary owing to heavy sporing of the sample—the other was plated after drying (series C and C<sub>1</sub>) (Table XV).

Table XV.

Sample	Period of incubation (days)	No. of plates	No. of colonies	Dilution
A	6	8	17.12 $\pm$ 1.1	1 in 20,480
A <sub>1</sub>	6	8	13.5 $\pm$ 0.9	1 in 20,480
B	6	8	251.87 $\pm$ 11.3	1 in 20,480
B <sub>1</sub>	6	8	149.12 $\pm$ 5.1	1 in 20,480
C	6	8	8.25 $\pm$ 0.5	1 in 655,360
C <sub>1</sub>	6	8	5.37 $\pm$ 0.6	1 in 655,360

It will be noticed that the number of colonies developing on plates from sample B<sub>1</sub> is not so great as the number developing from sample B. An analysis of the colonies showed, however, that the proportion of colonies of each species remained approximately the same, pointing to the fact that the depression is due to the suppression of mycelium in the dried sample for, if spores were killed, the proportion would tend to be irregular (Table XVI).

Table XVI.

Sample B				Sample B <sub>1</sub>			
Plates	Total	Analysis		Plates	Total	Analysis	
		<i>Verti-cillium</i>	<i>Peni-cillium</i>			<i>Verti-cillium</i>	<i>Peni-cillium</i>
1	228	19	Rest	1	155	10	Rest
2	239	40	"	2	162	12	"
3	318	30	"	3	147	13	"
4	244	27	"	4	161	8	"
5	285	25	"	5	153	13	"
6	233	38	"	6	153	10	"
7	241	35	"	7	118	11	"
8	227	20	"	8	139	11	"

It is interesting to note at this point that Ludwig<sup>(11)</sup>, experimenting with cotton seed infected by the anthracnose fungus *Glomerella gossypii*, found that "storage in a very dry atmosphere, e.g. in a desiccator over calcium chloride, was found to prolong the life of the fungus to a great extent whatever the preceding treatment of the seed."

The fact that in no case were the colonies entirely suppressed by drying may be due to one or two factors. In the first place, as lack of time has necessarily made this work preliminary in character, no attempt was made to measure degree of drying and it may be that fragments of mycelium still retain their vitality at the end of the three-day drying period. More probably, however, as the surface layers were included in the original sample of soil used, some spores of air forms

may have been present; if so, these would withstand the drying period and appear on the plates.

The results arrived at by the direct microscopic examination of soil seem to be in conflict with the idea here suggested—that fungi are present in the soil extensively, in fact practically entirely, in the mycelial condition. However, it is now well established that algae and protozoa are present in soils in numbers far exceeding those estimated by direct microscopic examination. Cutler<sup>(8)</sup> has shown that the factors governing the relation between the protozoa and the soil particles are those of surface action. The same factors are no doubt operative in the case, at least, of the unicellular algae and the acknowledged difficulty in demonstrating their presence by the direct method, although they may be present in the soil in large numbers, is thus explained.

The fine fungal threads forming a network round the soil particles would, for similar reasons, be equally difficult to demonstrate in quantity and so no idea of the mycelial distribution can be gained by a direct soil examination.

#### SUMMARY.

(1) Sterile soil was inoculated with a known quantity of spores of four different fungi, incubated at 9° C. and at intervals representative samples were plated out by the dilution method and an analysis of the plate population made. Results showed that high plate counts were not in any way connected with vegetative growth and supported Conn's idea that in such a case one is simply measuring the sporing capacity of the forms used.

(2) Samples of moist soil and of soil which had been dried in a vacuum desiccator over calcium chloride were plated out by the dilution method and the number of fungal colonies per plate compared. A marked decrease was noted with the dried sample. The reduced pressure was found to have no effect as drying under ordinary air-pressure gave comparable results.

(3) Suspensions in soil, and in sand, of fragmented mycelia and of a mixture of fungal spores, were in turn plated out directly and after drying. No colonies developed from the sample in the desiccator containing only mycelia, whereas the sample containing spores was in no way affected. It is suggested therefore that the decrease obtained after drying is due to the desiccation of the vegetative mycelium in the soil and since the reduction in the number of colonies per plate is very pro-

nounced after this treatment, it is thought that the normal fungal constituents of the soil are present extensively in the mycelial condition.

In conclusion I should like to acknowledge my indebtedness and gratitude to Sir John Russell for offering to me the hospitality of the Rothamsted Experimental Station and to Dr W. B. Brierley, Head of the Department of Mycology, for placing at my disposal all the facilities of the Department and for his encouragement and helpful criticism during my work at the Station.

### BIBLIOGRAPHY.

- (1) ALLISON, F. E. (1917). Biological Changes in Soil during Storage. *Soil Sci.* III.
- (2) BRIERLEY, W. B. (1923). The micro-organisms of the soil. Chaps. VII and VIII (Fungi). In Sir John Russell and others, *The Rothamsted Monographs on Agricultural Science*. Longmans Green & Co.
- (3) ——— JEWSON, S. T. and BRIERLEY, M. F. The Quantitative Study of Soil Fungi. *Transactions International Congress of Soil Science*. Washington, 1927.
- (4) BROWN, P. E. (1917). The importance of mold action in soils. *Science*, N.S. XLVI, No. 1182.
- (5) CHURCH, M. B. and THOM, C. (1921). Mold hyphae in Sugar and Soil. *Ibid.* LIV.
- (6) CONN, H. J. (1918). The microscopic study of bacteria and fungi in soil. *New York Agric. Expt. Sta. Tech. Bull.* 64.
- (7) ——— (1922). A microscopic method for demonstrating fungi and actinomycetes in soil. *Soil Sci.* XIV.
- (8) CUTLER, D. W. (1919). Observations on Soil Protozoa. *Journ. of Agric. Sci.* IX.
- (9) HARRINGTON, G. T. (1918). Resistance of Seeds to Desiccation. *Journ. Agric. Res.* XIV.
- (10) LEBEDJANTZEV, A. N. (1924). Drying of soil, as one of the natural factors in maintaining soil fertility. *Soil Sci.* XVIII.
- (11) LUDWIG, C. A. (1925). Studies with anthracnose infection in cotton seeds. *South Carolina Agric. Expt. Sta. Bull.* 222. (Abstract in *Rev. App. Myc.* v, 1926, 12.)
- (12) WAKSMAN, SELMAN A. (1916). Do fungi live and produce mycelium in the soil? *Science*, N.S. XLIV, No. 1131.
- (13) ——— (1918). The importance of mold action in the soil. *Soil Sci.* VI.
- (14) ——— (1922). A method for counting the number of fungi in the soil. *Journ. of Bacteriol.* VII.
- (15) ——— (1922). The growth of fungi in the soil. *Soil Sci.* XIV.
- (16) ——— (1917). Is there any fungus flora of the soil? *Ibid.* III.
- (17) WINOGRADSKY, S. (1926). Études sur la microbiologie du sol. *Ann. Inst. Past.* XL.

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# On the Influence of Light and of Glucose on the Growth of a Soil Alga.

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With nine Figures in the Text.

## I. INTRODUCTION.

THE relative importance of photosynthesis and of the absorption of organic food materials in the nutrition of the algae living in soil is a subject of vital interest in the study of these organisms. For while the building up of organic material by photosynthesis is possible on the surface of the soil, and all of the species so far isolated are capable of growing in aqueous media containing mineral salts only, yet the addition of certain organic compounds to the medium does tend to increase the growth of many of the species even in the light, while in complete darkness photosynthesis is quite impossible. The question therefore arises as to which of these two types of nutrition is the dominant one; is, for example, the visible stratum of algae which appears in damp weather on the surface of the soil produced mainly as a result of photosynthesis, or is it largely built up from organic substances absorbed from the soil? In other words, does such a stratum of algae contribute to the fertility of the soil by adding to it relatively large amounts of organic matter, or does it lessen the fertility by immobilizing organic and nitrogenous compounds that would otherwise be available for the crop?

A careful study of the literature dealing with the nutrition of the semi-saprophytic algae shows that except in the case of lichen gonidia, the most highly specialized perhaps of all algae, which only grow vigorously in the presence of peptone (Artari, Chodat, &c.), no clear experimental evidence has as yet been obtained on this point, and the general opinion seems to be that both processes are carried on simultaneously, even in the light.

In a recent paper (1) the writer described a method by which the relative rate of increase in bulk of a unicellular alga in pure culture, under

absolutely controlled conditions, could be measured with a fairly high degree of accuracy, and applied it to a study of the nutrition of the soil alga, *Scenedesmus costulatus*, Chod., var. *chlorelloides*. The rate of growth was taken as a measure of the metabolism of the organism in any given set of conditions, and by varying one condition at a time the quantitative effect of that condition on the metabolism of the alga could be determined. Details were given of two experiments which gave results that are relevant to the present subject. In the first (Part II, Section B, iii b), parallel cultures in a medium containing mineral salts only were compared with a control culture to which 1 per cent. glucose had been added, all the cultures being equally illuminated and continuously aerated; and it was found that the calculated figures for the rates of growth in the parallel mineral salts cultures were respectively 0.266 and 0.263 (almost identical), while that for the glucose culture was 0.434. That is to say, the rate of growth due to photosynthesis in the mineral salts medium was roughly 60 per cent. of that when glucose was present.

In the second experiment (Part II, Section B, iv), two cultures containing 1 per cent. glucose in complete darkness were compared with an exactly similar culture placed in the light; and though the conditions of this experiment were less satisfactory than those of the other experiments, the interesting fact was noted that the rate of growth of the more satisfactory culture in the dark was roughly 40 per cent. of that in the same medium in the light. The two experiments taken together seemed to indicate that the rate of growth of the alga in the glucose medium in the light was the additive result of two processes, viz. photosynthesis about 60 per cent. and the direct assimilation of glucose about 40 per cent. This led to the suggestion that probably 'the organism in the glucose medium in the light carries on normal photosynthesis and the assimilation of glucose independently of one another' and simultaneously; though it was pointed out that further evidence would be required before the fact could be regarded as established.

This line of investigation seemed to be so promising and the method so suitable for the purpose that it has been followed up by a number of other experiments designed to throw further light on the subject, using the same organism and based on the same method; the details of some of these experiments are described in the following pages, and show that the subject is more complicated than appears at first sight.

The salient points of the method may be briefly recapitulated. (For full details and precautions see earlier paper.) The alga is grown in liquid media under constant external conditions in special culture flasks from which, after vigorous shaking, a daily sample is taken through the side arm. The number of cells per cubic millimetre of medium in the daily samples is counted under the microscope in a Bürker haemocytometer, and camera

lucida drawings are made of fifty consecutive cells. From these data, the cells in liquid media being approximately spherical, the daily volume of algal protoplasm per cubic millimetre of medium is calculated. It has been shown that the alga, under the conditions described, generally increases in bulk for about ten days according to the compound interest law of growth; so that the logarithms of the calculated values for the volume of algal protoplasm when plotted against the number of days' growth are found to lie approximately along a straight line. The angle which this straight line makes with the horizontal axis gives a measure of the average relative rate of growth of the alga during this period, and may be determined by calculating algebraically the equation to the straight line which most nearly fits the observed logarithmic values. The coefficient of  $x$  in this equation, being the tangent of the angle which the line makes with the horizontal axis, is used as a measure of this angle, and is a convenient figure to use in comparing the rates of growth of the alga under different conditions; for in all experiments so far carried out, parallel cultures under identical conditions have been found to give approximately the same value for the coefficient of  $x$ .

In attempting to assess the effect of any given condition on the growth of the alga, it is absolutely essential that the cultures to be compared should be grown under conditions that are otherwise identical. In the experiments to be described, temperature was controlled by growing the cultures with the bulbs of the flasks completely immersed in a large water-bath kept at  $24.5^{\circ}$  C.; the source of light, except where otherwise stated, was a single opal 60-watt bulb immersed in the water-bath to the same depth as the cultures; and a uniform source of air supply for continuous aseptic aeration was secured for the more critical experiments by means of the special apparatus illustrated in Fig. 3 and described fully in Section III.

## II. ARE PHOTOSYNTHESIS AND THE ASSIMILATION OF GLUCOSE INDEPENDENT AND SIMULTANEOUS?

An attempt to obtain a definite answer to this question was made by combining in a single experiment the two sets of conditions investigated in the two experiments mentioned in Section I, i.e. by comparing the effects of cutting out light and glucose separately in cultures derived from the same algal stock.

*Experiment I.* Two similar aluminium water-baths were adjusted to keep at the same temperature,  $24.5^{\circ}$  C., by means of sensitive thermo-regulators (extreme variation about the mean not more than  $0.05^{\circ}$  C.), and were placed, one at a distance of about 6 ft. from the window of a north room, and the other in a specially constructed dark room where complete darkness was retained by surrounding the base of the water-bath and the

Bunsen burner attached to the thermo-regulator with a closely fitting cylinder of black paper.

Four cultures were set up, three (A, B, and C) containing mineral salts + 1 per cent. glucose and the fourth (D) containing mineral salts alone, and were inoculated with enough of a counted suspension of cells to give two cells per c.mm. of medium in the cultures. Cultures A and B were fitted with attachments for continuous aseptic aeration and placed in the bath in the dark room; flasks C and D were similarly fitted and placed in the bath in the light. In this way it was possible to obtain information in regard to the behaviour of the parallel Cultures A and B in the dark, to the influence of glucose on the growth of the alga in the light by comparing Cultures C<sup>1</sup> and D,<sup>1</sup> and to the influence of light on the growth of the alga in a glucose medium by comparing the mean of Cultures A and B with Culture C.

If there were any truth in the suggestion put forward in the earlier paper, that photosynthesis and the assimilation of glucose are independent processes carried on simultaneously, one would expect to find, with such an arrangement of the cultures as that above, that the rate of growth in Culture C, to which both photosynthesis and glucose were available, would be approximately equal to the sum of the rates in D, restricted to photosynthesis, and the mean of A and B, restricted to the assimilation of glucose.

The logarithmic values of the bulk of algal protoplasm in the four cultures are given in Table I and plotted in Fig. 1.

It is seen by inspection of the diagram that the observed values for Culture C (glucose in the light) in the earlier part of the experiment lie very closely along the straight line calculated to fit most nearly the first eleven points, after which the relative rate of increase gradually falls off during the rest of the experiment. The growth of this culture was thus exactly typical of what has always been observed in cultures containing glucose in the light. The calculated equation to the line of nearest fit is  $y = 0.314x + 2.574$ , so that the slope of the line, i.e. the relative rate of increase of the alga, is represented by the figure 0.314.

The parallel cultures in the dark gave results that are astonishingly closely in agreement, but it is questionable whether the observed points can really be regarded as being represented by a straight line, at any rate after the first week's growth. On the contrary, there is a suggestion of 4-daily pulsations of increase in bulk, corresponding to successive reproductive phases, coupled with a gradual falling-off in the average relative rate of growth almost from the beginning of the experiment. Full details of the measurements are given in Table II for Culture B, in which the first point of fluctuation (day 4) is not evident. Up to and including day 7 of the experiment there was a steady increase both in the size of the cells and in their

<sup>1</sup> Parallel cultures under each of these two conditions were already known to behave identically, hence a single culture of each kind was enough for comparison.

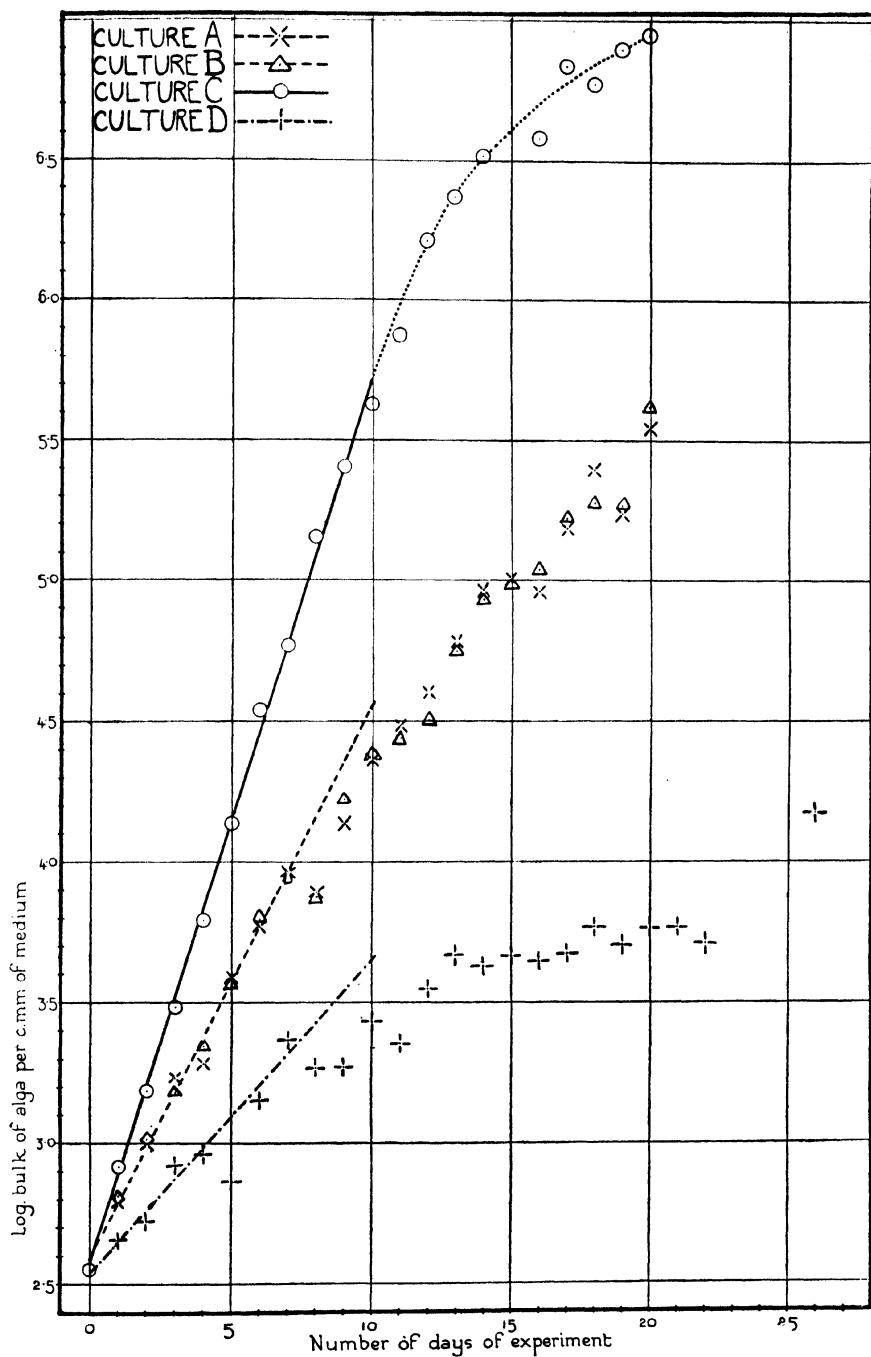


FIG. 1. Diagram showing relative rates of growth of *Scenedesmus* sp. in a medium containing mineral salts alone (Culture D), and in the same + 1 per cent. glucose in diffuse daylight (Culture C), and in complete darkness (Cultures A and B). For explanation see text.

numbers. On days 8 and 9 there was a considerable increase in numbers, which was reflected in a significant decrease in the average size of the cells, largely owing to the presence of a smaller proportion of very large cells due to their multiplication. Then followed a second steady rise in size of the cells up to day 15, with considerable increase in numbers on days 9 and 13,

TABLE I.

*Bulk of Algal Protoplasm per c.mm. of Medium (log. values) in Aerated Cultures containing Mineral Salts alone and the same + 1 per cent. Glucose in Diffuse Daylight and in Complete Darkness.*

<i>Day of Experiment.</i>	<i>Cultures in Darkness.</i>		<i>Cultures in Diffuse Daylight.</i>	
	<i>Glucose Medium.</i>		<i>Glucose Medium.</i>	<i>Mineral Salts alone.</i>
	<i>Culture A.</i>	<i>Culture B.</i>	<i>Culture C.</i>	<i>Culture D.</i>
0	2.5532	2.5532	2.5532	2.5532
1	2.7937	2.8147	2.9191	2.6571
2	3.0004	3.0165	3.1878	2.7235
3	3.2352	3.1835	3.4833	2.9217
4	3.2833	3.3448	3.7918	2.9619
5	3.5915	3.5706	4.1390	2.8632
6	3.7701	3.7994	4.5403	3.1550
7	3.9640	3.9508	4.7701	3.3068
8	3.8935	3.8767	5.1587	3.2704
9	4.1377	4.2225	5.4977	3.2716
10	4.3623	4.3742	5.6299	3.4323
11	4.4854	4.4399	5.8775	3.3581
12	4.6025	4.5031	6.2138	3.5475
13	4.7822	4.7506	6.3674	3.6665
14	4.9681	4.9326	6.5144	3.6264
15	5.0068	4.9900	—	3.6653
16	4.9630	5.0422	6.5786	3.6454
17	5.1897	5.2261	6.8360	3.6760
18	5.3973	5.2790	6.7705	3.7680
19	5.2397	5.2697	6.8984	3.7040
20	5.5424	5.6202	6.9451	3.7638
21	—	—	—	3.7657
22	—	—	—	3.7090
26	—	—	—	4.1761

and so on. This gradual increase in size of the cells is a constant feature of cultures grown in the dark, and is one of their most striking characteristics; the extreme variation in average cell diameter for Culture C (with glucose in the light) was only  $6.1\mu$ – $8.4\mu$ , while the fluctuation in Culture D was even less,  $6.9\mu$ – $8.3\mu$ . One of the effects of glucose in the dark therefore appears to be to delay multiplication, without, however, imposing any serious restrictions on the possible dimensions of the individual cells. Occasional subspherical cells have been observed of which the diameter was up to  $32\mu$ , while cells of  $25\mu$  diameter were comparatively common. In contrast to this, the maximum size observed in Cultures C and D was  $12\mu$  diameter, and this was only occasionally attained.

Coupled with this great increase in size of cell was observed a certain amount of abnormality in method of multiplication (Fig. 2) similar to that

already described and figured in a medium containing fructose (1). For whereas normal multiplication into eight or more equal aplanospores was quite often observed, yet a fair proportion of cells multiplied abnormally by means of budding or by producing aplanospores of very disproportionate sizes. The presence in the cultures of cells of this type greatly increased the difficulty of obtaining accurate data in regard to both number and size of the cells, and it is possible that the apparent rhythm in the bulk curves may be due to experimental error. On the other hand, since the fluctuations correspond to successive reproductive phases, they may indicate a slowing down of vegetative growth preparatory to multiplication such as would be obliterated by overlapping in the more rapidly multiplying cultures in the light.



FIG. 2. Enlarged cells of *Scenedesmus costulatus*, var. *chlorelloides*, undergoing abnormal multiplication after growing in complete darkness for five weeks in a medium containing mineral salts + 1 per cent. glucose.

TABLE II.

*Experimental Details for Culture B. Glucose Medium in Complete Darkness.*

Day of Experiment.	Average Diameter of 50 cells. ( $\mu$ ).	Average $n^3$ . ( $\mu^3$ ).	No. of Cells per c.mm.	Bulk ( $\mu^3$ ) per c.mm.	Log. Bulk.
0	6.9	42.66	2	357	2.5532
1	7.6	57.72	2.7	653	2.8147
2	8.7	88.59	2.8	1,039	3.0165
3	9.2	107.13	3.4	1,526	3.1835
4	9.2	112.35	4.7	2,212	3.3448
5	9.5	128.75	6.9	3,721	3.5706
6	11.0	192.87	7.8	6,302	3.7994
7	12.2	273.59	7.6	8,928	3.9508
8	10.2	159.05	11.3	7,528	3.8767
9	10.8	189.75	21	16,690	4.2225
10	10.5	188.33	30	23,670	4.3742
11	10.9	187.86	35	27,540	4.4399
12	11.6	245.27	31	31,850	4.5031
13	12	268.87	50	56,310	4.7506
14	12.2	329.67	62	85,620	4.9326
15	12.1	271.3	86	97,730	4.9900
16	11.4	232.76	113	110,200	5.0422
17	12.2	269.62	149	168,300	5.2261
18	10.7	226.92	200	190,100	5.2790
19	9.3	132.21	336	186,100	5.2697
20	10.8	204.47	487	417,100	5.6202

Whether these fluctuations are genuine rhythms of growth, or whether they are merely the outcome of experimental error, there is no question that

for the first week's growth the straight line of nearest fit to the observed values does represent fairly accurately what is happening on the average in the two cultures, for the equations calculated separately for the two sets of values are almost identical, viz.:

$$y = 0.197x + 2.584 \quad (\text{Culture A})$$

$$\text{and } y = 0.197x + 2.588 \quad (\text{Culture B}),$$

giving for both cultures an average relative rate of increase of 0.197, or 63 per cent. of that in the same medium in the light.

The values obtained for Culture D, in mineral salts alone in the light, are very much less regularly disposed than those obtained in earlier experiments under similar conditions; and it was realized after several days that though the sun was shining brightly outside during most of the experiment, yet the intensity of the light within the water-bath in the north room was insufficient for really satisfactory growth by means of photosynthesis, much less in fact than had been obtained in the earlier experiments in a laboratory with an eastern aspect. The first eight points, omitting the sixth, which was recorded as probably subject to error at the time the observations were made, are distributed evenly about the calculated straight line of which the equation is

$$y = 0.111x + 2.54;$$

so that the average rate of growth during the first week is probably represented fairly nearly by the figure 0.111, which is 35 per cent. of the rate in the glucose medium in the light.

The experiment gives the very striking result, therefore, that if the rate of growth of the alga in a glucose medium in the light (in which both photosynthesis and the direct absorption of glucose are available) be expressed as 100, then that in mineral salts alone in the light (confined to photosynthesis) was about 35, while that in the glucose medium in the dark (confined to the direct assimilation of glucose) was 63. That is to say, the sum (98) of the rates of growth due to the two processes separately is approximately equal to the rate of growth (100) in the culture where both processes were going on at the same time, the difference being well within the limits of experimental error.

The results of this experiment are therefore in direct support of the suggested hypothesis that the organism under investigation is capable of carrying on both photosynthesis and the absorption of organic substances simultaneously without either process affecting the other. It was felt, however, that further corroboration was required under conditions more favourable to photosynthesis; the experiment was therefore repeated under conditions in which continuous electric light served for illumination and an equal supply of carbon dioxide was ensured to all four cultures.

*Experiment II.* The apparatus was set up in a single large water-

bath in the dark room, at a constant temperature of  $24.5^{\circ}\text{C}$ . A 60-watt, gas-filled, opal electric light bulb, the metal parts of which were carefully protected by insulating tape, was submerged centrally in a beaker of running water. This was contained in a larger empty glass cylinder weighted at the bottom with sand, and the whole immersed on one side of the water-bath (see Fig. 3). In this way a lateral illumination of the culture flasks was secured by means of a beam of light entering the water at the same angle for all flasks at equal depths and at equal radial distances from the source of light, while the running water and surrounding air-chamber prevented the heat from the submerged bulb from affecting the temperature of the water-bath. Flask A, containing mineral salts alone, and flask B, containing in addition 1 per cent. glucose, were placed in the bath at a radial distance of 16 cm. from the centre of the bulb.<sup>1</sup> Complete darkness for Cultures C and D, in mineral salts + 1 per cent. glucose, was secured by growing them in two culture flasks of which the bulb, neck, and side arm excluding the tap had been completely covered with a thick coating of electrolytically deposited copper, so that they could be kept at the same temperatures as Cultures A and B by being immersed in the same bath without any risk of penetration by light.

In order to ensure that the four cultures received equal amounts of carbon dioxide for possible photosynthesis, they were all connected by means of glass and rubber tubing to a single inlet tube, and a common filter-pump served to draw the stream of air through the cultures. The arrangement of the tubes when assembled resembled, with a few minor differences, that described in detail and illustrated in Section III.

Observations were recorded in this experiment for nine days only; but, apart from the fact that Culture C in the dark became badly contaminated after the fourth day and had, therefore, to be disregarded owing to arrested growth, the curves obtained for the cultures showed no departure of the organism from its normal mode of behaviour under these conditions; only the logarithmic values recorded for Culture D lay exceptionally close to the straight line calculated to fit them, and there was no suggestion of rhythm in the rate of increase during this period of growth.

Cultures A and B exposed to light grew very much more rapidly than the corresponding cultures in the previous experiment, especially that containing mineral salts only, so that the conditions of the experiment were evidently quite suitable for normal photosynthesis.

The logarithmic values of the observed points are represented closely by the straight lines of which the equations are as follow:

(i)  $y = 0.393x + 2.57$  for Culture A, in mineral salts alone in the light.

<sup>1</sup> Parallel cultures at equal radial distances from this source of light had previously been found to grow at approximately the same rates.

(ii)  $y = 0.47x + 2.6$  for Culture B, in mineral salts + 1 per cent. glucose in the light.

(iii)  $y = 0.209x + 2.67$  for Culture D, in mineral salts + 1 per cent. glucose in the dark.

Hence, the relative rate of growth in the glucose medium in the light (0.47) being computed at 100 per cent., that due to photosynthesis alone in Culture A (0.393) is 83.6 per cent., while that due to the absorption of glucose alone in the dark (0.209) is 44.5 per cent.

Now the rate of growth in Culture D in the dark is very little different from the rate of growth in the corresponding parallel cultures in the previous experiment (0.197), and differs from the average obtained for eight similar cultures (0.207) by only 1 per cent. It therefore probably represents the average capacity of the alga to grow by means of the direct absorption of glucose under the conditions investigated. But since the capacity of the alga to grow under purely photosynthetic conditions is estimated at 83.6 per cent. of that when glucose is also available for direct assimilation, then in the latter case either the rate of absorption of glucose has been cut down to nearly one-third of that possible in the dark, or conversely the photosynthetic capacity of the alga may have been reduced to about two-thirds by the presence of directly available glucose; or there may have been mutual interference between the two processes.

The directly additive results, therefore, obtained in the earlier experiments appear to have been due to the accident that the cultures had been grown under conditions of relatively low light intensity, which reduced the rate of growth due to photosynthesis to a comparatively low figure.

The apparently contradictory results in the two sets of conditions have led to a further series of experiments in an attempt to assess the influence of light of varying intensity upon the growth of the alga, both under purely photosynthetic conditions and in the presence of directly assimilable glucose. It was hoped at the outset that a simple numerical relationship would be found to hold between the rate of growth of the alga and the intensity of the light. This, however, is not so; the relationship is not sufficiently simple for the determination of its character to be possible on the basis of the data available.

This is largely due to the difficulty of estimating accurately the intensity of the effective light within a given culture, and therefore of correlating the results of separate experiments in which different electric-light bulbs necessarily had to be used; and though the results obtained within a single experiment are comparable, yet the physical impossibility of dealing single-handed in a satisfactory manner with more than four cultures at a time renders the data inadequate for mathematical treatment. Certain facts which may be expressed in general terms, however, may be deduced from

the data, and these are presented by describing in detail two representative experiments.

### III. ON THE EFFECT OF VARYING LIGHT INTENSITY ON THE RATE OF GROWTH OF THE ALGA.

#### (1) *Apparatus.*

During the course of the experiments under discussion, a number of difficulties were encountered which had to be overcome in order to ensure reliability of the results. These were chiefly concerned with keeping the cultures uncontaminated during continuous aeration and with providing all cultures with an equal supply of air from the same source. The chief cause of the difficulties was the atmosphere of the dark room in which the experiments were conducted, which, in spite of the continuous working of an electric fan on the outer end of the ventilation tunnel, became completely saturated with moisture by evaporation from the rather extensive surface of the water-bath. When air was drawn through the cultures, some of this moisture condensed in the sterile cotton-wool plugs of the inlet tubes, providing a very favourable site for the growth of contaminating organisms; while the exit plugs, after the passage of the air through the slightly warm cultures, became so sodden as occasionally to prevent completely the suction of the air. A number of devices were therefore adopted in order to combat these troubles as far as possible, and an apparatus was gradually built up, of which the final form is illustrated in Fig. 3.

The bulbs of the culture flasks described in the earlier paper were immersed in a large circular water-bath at  $24.5^{\circ}\text{C.}$ , painted inside with black paint to prevent reflections from its surface. The neck of each flask was fitted with a two-holed rubber stopper through which passed (*a*) a long inlet tube ( $J_1$ ) that was attached to a two-bulbed calcium chloride tube ( $H_1$ ) full of cotton-wool outside the flask, and dipped to the bottom of the culture fluid, where it was bent at right angles and drawn out to an aperture of about  $\frac{1}{16}$  in. diameter, and (*b*) a short exit tube ( $J_1$ ) bent towards the side of the neck away from the side-arm used for sampling. The outer end of the exit tube was bent twice at right angles and attached by means of a long piece of flexible rubber tubing (to facilitate the shaking of the culture) to an apparatus ( $K_1$ ) in which the moisture in the air current was condensed before it reached the sterile exit plug ( $M_1$ ). The condensing apparatus had to be capable of sterilization in the autoclave, and consisted of a calcium chloride tube packed loosely with glass-wool to increase condensation and fitted into one hole of the rubber stopper of a small Erlenmeyer flask; through the second hole in the stopper passed an exit tube bent at right angles and attached at its outer end to a calcium chloride tube ( $M_1$ ) which was filled with cotton-wool and fixed beneath the bath, where the temperature was

slightly higher than that of the surrounding room. The stopper of the Erlenmeyer flask was made completely air- and water-tight with a coating of paraffin wax and immersed in a glass cylinder full of slowly running tap-water, being kept in position by means of a leaden collar. The moisture in the air stream condensed on the glass-wool and in the Erlenmeyer flask, at the bottom of which it gradually collected until, at the end of an experiment lasting two or three weeks, there was as much as 5–10 c.c. of water; and the sterile exit plug under the bath consequently remained quite dry. It was connected by means of glass and rubber tubing with the exit tubes of the other flasks, and the common exit tube (N) was then attached to a single filter-pump, by means of which air was drawn through the apparatus. The rate of flow of the air current through the medium in the culture flasks was equalized by means of screw clips on the rubber connexions.

In order to ensure a uniform supply of air to all the cultures of an experiment, the inlet tubes ( $H_1$ ,  $H_2$ , &c.) were connected with one another by means of glass and rubber tubing, as shown in the diagram, to form a common inlet tube, the entrance to which was protected by means of a large calcium chloride tube (G) filled with cotton-wool. This in its turn was connected to an apparatus for washing and drying the incoming stream of air (A–F). The air was first filtered by passing through a small calcium chloride tube, loosely plugged with cotton-wool and fitted through one hole in the rubber stopper of a small empty Erlenmeyer safety flask (A) which was attached by a tube through the second hole in its stopper to a washing bottle half filled with a 1 per cent. solution of sodium bicarbonate (B). After being washed in the bicarbonate solution, the moist air passed through a second empty safety flask (C) into a second washing bottle (D) one-third filled with concentrated sulphuric acid, whence it passed in a relatively dry condition. Owing to the large amount of water absorbed by the sulphuric acid, the washing bottle gradually became filled with liquid, and a certain amount of the acid was carried over by the air stream into the safety flask E in the form of a fine spray. To make quite sure that no acid could be carried into the cultures, the air stream was finally drawn through a bulb U-tube filled with small chips of marble (F), before being allowed to enter the common inlet tube at G. The value of this last precaution was observed at the end of an experiment, when the surface of the nearer marble chips was found to be coated with a layer of calcium sulphate.

As mentioned above, the condensing apparatus ( $K_1$ ) was cooled by means of tap-water running continuously through the outer glass cylinder. Owing to frequent fluctuations in the pressure of the main water-supply, it was found necessary to introduce a constant-level tank to serve as supply to the condensing cylinders to prevent their constantly overflowing. This took the form of a large aspirator bottle (P) filled to overflowing directly from a tap by means of a glass tube passing through one hole of a rubber

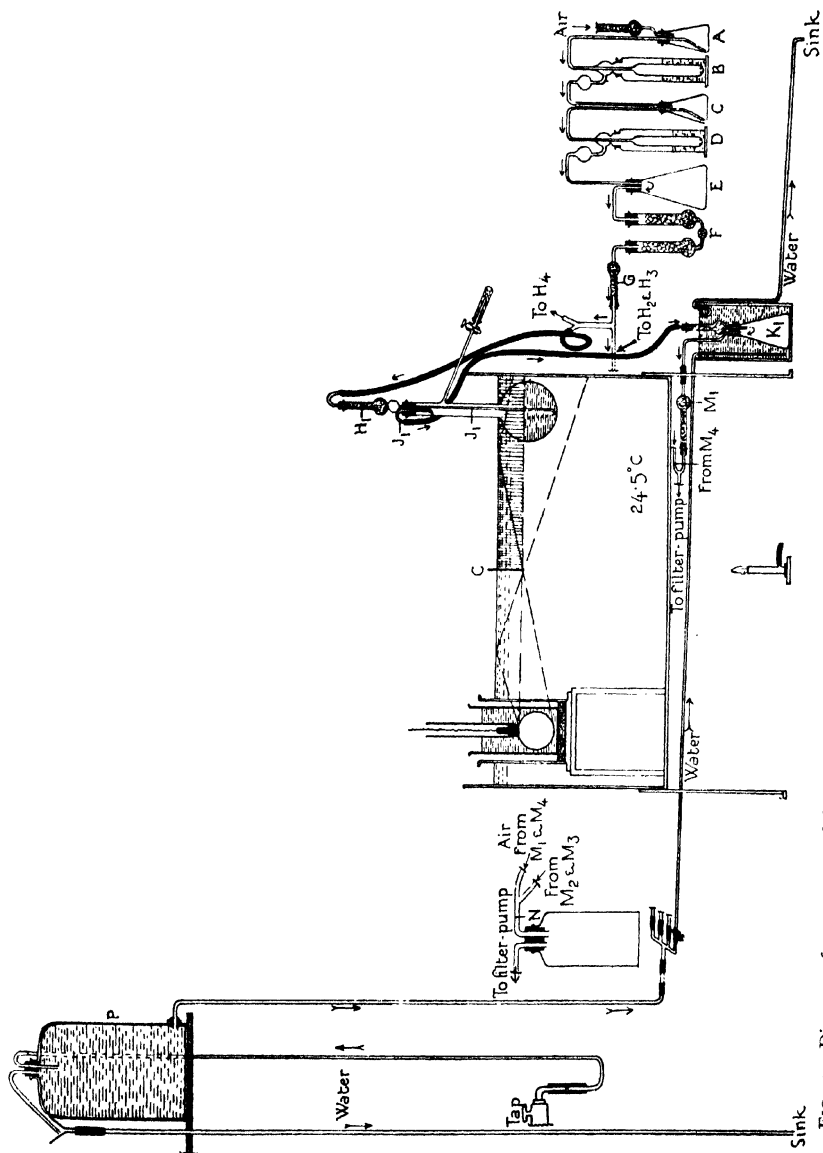


FIG. 3. Diagram of apparatus used for comparing the growth of cultures of algae in liquid media under aseptic conditions with constant temperature, controlled illumination, and continuous aeration from a single source. For explanation see text.

stopper in its neck. A wide bent exit tube through the second hole of the stopper served to carry away the slowly overflowing water, while a single glass tube fixed with a rubber stopper into the tubulure at the base of the bottle was connected to a branched glass tube, as shown in the diagram, and served all of the four condensing cylinders with a continuous supply of running water at a constant pressure. The rate of inflow for each condenser was regulated by means of a screw clip on its rubber connexion to be just sufficient to secure a continuous stream of alternate water and air bubbles in the narrow siphon tube provided to carry away the overflow from the condenser to the sink.

The source of illumination of the cultures was a 60-watt, opal electric bulb submerged in the bath as described on p. 325 for the last experiment; but, whereas in the earlier investigation comparison was desired between cultures grown under conditions of equal illumination which could be easily secured by placing them at equal distances from the bulb, in the present series of experiments different intensities of illumination were required and could be secured only by placing the flasks at different distances from the source of light. Consultation with a number of physicists elicited the fact that methods for measuring relative intensities of light under conditions such as those existing in these experiments have not been worked out, and that the rule that the intensity of the light varies inversely as the square of the distance from its source, though at best only an approximation, probably gives as accurate a measure as any other more elaborate method. After a few trials an added complication was realized, in that the water-air surface of the bath was acting as a mirror, and providing, by total reflection to the more oblique rays of light, a higher light intensity to the more distantly placed flasks than was their due. This difficulty was obviated by placing across the bath vertical barriers to cut off the upper beams of light, half-way between the bulb and the culture flasks, and at right angles to the direction of the rays. Each barrier ( $O_1$  and  $O_2$ ) consisted of a strip of copper  $3\frac{1}{2}$  in. wide, of which half an inch projected above the surface of the water and the rest extended below to the level of the top of the culture fluid and of the spherical part of the electric bulb. Beams of light striking the surface of water in front of these barriers, even if totally reflected, would not strike the culture flasks directly and would be absorbed by the black surface of the bath. Rays striking the surface of the water beyond the barrier would be reflected back at too oblique an angle to enter the culture fluid directly, and would not therefore cause a serious error in the intensity of the light within the culture fluid (see Fig. 3, where beams of light are represented by sparsely broken lines, and shadows by vertical shading).

## (2) Experiment III.

This experiment is representative of several carried out in order to ascertain the effect of varying light intensity on the rate of growth under purely photosynthetic conditions. Four exactly similar cultures containing a mineral salts medium only were inoculated with enough of a suspension of the alga to give two cells per c.c. of medium in each flask. They were

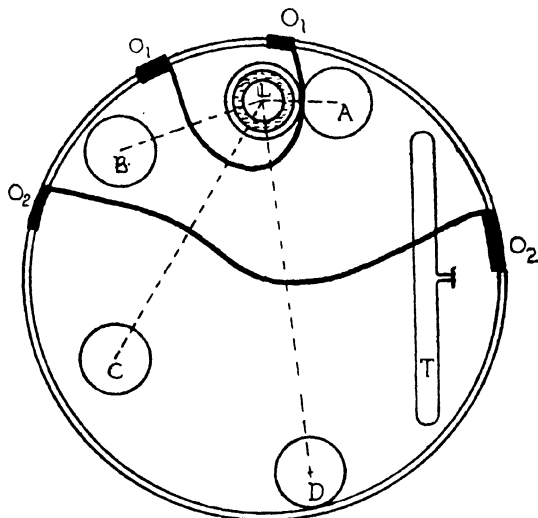


FIG. 4. Ground plan of the water-bath to show disposition of cultures in Experiment III. L, source of light; A, B, C, D, similar cultures at distances 3.5, 7, 14, and 17.5 inches respectively from centre of L: O<sub>1</sub> and O<sub>2</sub> barriers to prevent surface reflections; T, bulb of thermo-regulator.

then placed in the bath in the positions shown in Fig. 4, at distances  $3\frac{1}{2}$  in. (A), 7 in. (B), 14 in. (C), and  $17\frac{1}{2}$  in. (D) from the centre of the source of light (L), so that the relative intensities of light in the four flasks were approximately  $I$ ,  $I/4$ ,  $I/16$ , and  $I/25$  respectively, while no flask interfered in any way with the light obtained by any other. The position of the two light screens is indicated by the two heavy curved lines across the bath. Daily samples were taken for eleven days, yielding the results given in Table III and plotted in Fig. 5.

The straight lines of nearest fit to the observed values in the four cultures are expressed algebraically by the following equations:

- (i)  $y = 0.459x + 2.54$  for Culture A, with relative light intensity of  $I$ ; i.e. growth rate is 0.459, or 100 per cent.
- (ii)  $y = 0.351x + 2.555$  for Culture B, with relative light intensity of  $I/4$ ; i.e. growth rate is 0.351, or 76 per cent. of A.

(iii)  $y = 0.143x + 2.593$  for Culture C, with relative light intensity of  $1/16$  or  $1/4$  B; i. e. growth rate is 0.143, or 31 per cent. of A, or 41 per cent. of B.

(iv)  $y = 0.103x + 2.611$  for Culture D, with relative light intensity of  $1/25$ ; i. e. growth rate is 0.103, or 22 per cent. of A.

TABLE III.

*Bulk of Algal Protoplasm per cmm. of Medium (log. values) in Aerated Cultures containing Mineral Salts alone under Different Intensities of Light.*

Day of Experiment.	Culture A. Intensity 1.	Culture B. Intensity $1/4$ .	Culture C. Intensity $1/16$ .	Culture D. Intensity $1/25$ .
0	2.5999	2.5999	2.5999	2.5999
1	3.0115	2.8142	2.7474	2.7694
2	3.3395	3.2435	2.8573	2.8215
3	3.8509	3.6238	3.0140	2.8965
4	4.4544	3.9418	3.1547	2.8960
5	4.8577	4.4075	3.3173	3.1380
6	5.3049	4.6574	3.4276	3.2659
7	5.7330	5.0245	3.5734	3.4094
8	5.8679	5.3608	3.8302	3.3888
9	6.0249	5.6751	3.8370	3.6093
10	6.0823	5.8998	4.0464	3.6706
11	6.2450	5.9969	4.1467	3.6691

An examination of these results reveals the fact that increasing light intensity does increase very considerably the rate of growth due to photosynthesis when there is a uniform supply of carbon dioxide in all the flasks; but that with low intensities the effect of increase in light is more marked than with higher intensities. For instance, in the Cultures C, B, and A the light intensities were roughly in the proportions 1 : 4 : 16, while the rates of growth were 0.143, 0.351, and 0.459 respectively; that is to say, increasing the intensity of the light four times between Cultures C and B has increased the rate of growth about 2.5 times, while increasing the intensity four times between Cultures B and A has only increased the rate of growth 1.3 times, or about half as much as between Cultures C and B.

This phenomenon has been observed constantly in these experiments, indicating that there is only a limited extent to which the growth rate may be increased by increasing the intensity of the illumination under the conditions imposed, and that above a certain optimum intensity the light loses progressively its efficiency in promoting photosynthesis. It is possible, of course, that some external limiting factor comes into play in the higher light intensities, such as an insufficient supply of carbon dioxide for increased photosynthesis, a factor which has so far not been investigated; but in view of the results obtained from other experiments to be described later, it seems more likely that the falling-off is due to the working of some internal limiting factor in the cells, which restricts the increase of growth rate of the alga beyond an optimum value of about 0.475 under the conditions of these experiments.

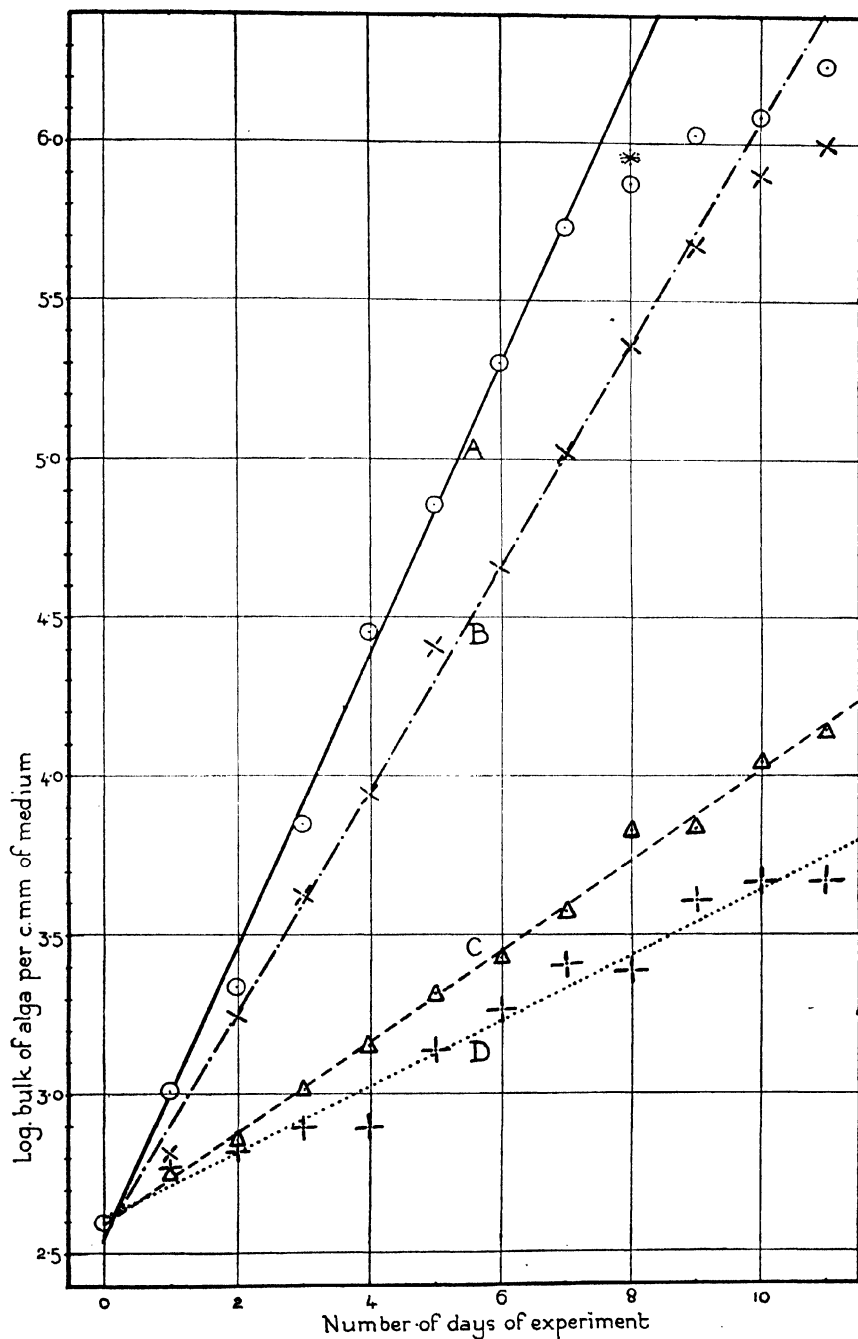


FIG. 6. Diagram showing growth rates of *Scenedesmus* sp. in a mineral salts medium under illumination of different intensities (Experiment III). Culture A, intensity  $I$ ; Culture B, intensity  $I/4$ ; Culture C, intensity  $I/16$ ; Culture D, intensity  $I/25$ . For explanation see text.

On the other hand, there is some evidence to indicate that with intensities of light below a certain standard the rate of increase in growth rate is roughly proportional to the rate of increase in light intensity. This is suggested by the curve drawn in Fig. 6, which was obtained by plotting the logarithms of the growth rates against the logarithms of the light intensities (see Table IV). Here the points representing Cultures D, C, and B lie approximately on a straight line, whereas that for Culture A shows a considerable falling-off below the line; while the general shape of the curve suggests that the illumination in Culture B was not far from the optimum intensity for its effective use in photosynthesis. The data are unfortunately too meagre, however, to regard these as more than tentative suggestions.

TABLE IV.

*Data for Fig. 6.*

<i>Culture.</i>	<i>Relative Growth Rate.</i>	<i>Log. Rel. Growth Rate.</i>	<i>Distance from Light.</i> Inches.	<i>Intensity of Illumination.</i>	<i>Log. Intensity.</i>
A	0.459	$\bar{1}.6561$	$3\frac{1}{2} \times 1$	$I$ or 25	1.3979
B	0.351	$\bar{1}.5453$	$3\frac{1}{2} \times 2$	$I/4$ or 6.25	0.7959
C	0.143	$\bar{1}.1553$	$3\frac{1}{2} \times 4$	$I/16$ or 1.5625	0.1938
D	0.103	$\bar{1}.0128$	$3\frac{1}{2} \times 5$	$I/25$ or 1	0.0000

Further, there appears to be a limit of low light intensity below which photosynthesis cannot effectively take place. This is shown by the fact that whereas the logarithmic values for the bulk of algal protoplasm in Cultures B and C are unquestionably capable of representation as straight lines, with extremely slight deviation of the observed points, yet the growth of Culture D is obviously subject to periodic fluctuations in rate similar to those observed in the parallel cultures with glucose in the dark, and the straight line can only be regarded as an average expression of the changes in the culture during the experiment. Fluctuations in growth rate of this type have been observed in a number of cultures, especially in those grown with a low illumination or in complete darkness, and appear to be an indication that the organism is growing under conditions not entirely favourable to its satisfactory development. Since the intensity of illumination in Culture C was only roughly one and a half times that in Culture D, there seems to be a very narrow range of illumination within which the phenomenon appears; this is borne out by the results of other experiments.

Finally, the rapid falling off in growth of Culture A after the eighth day had probably no connexion with the normal curve of increase, but was probably due to the growth of a contaminating fungus which made its first appearance on the ninth day, and increased so vigorously as to compete seriously with the further growth of the alga.

(3) *Experiment IV.*

A number of experiments were carried out to test the effect of varying light intensity on the growth of the alga when glucose was present in the medium. The one described below was conducted similarly to the last experiment, except that 1 per cent. of glucose was added to the medium and that Culture D was grown in a copper-covered flask in complete dark-

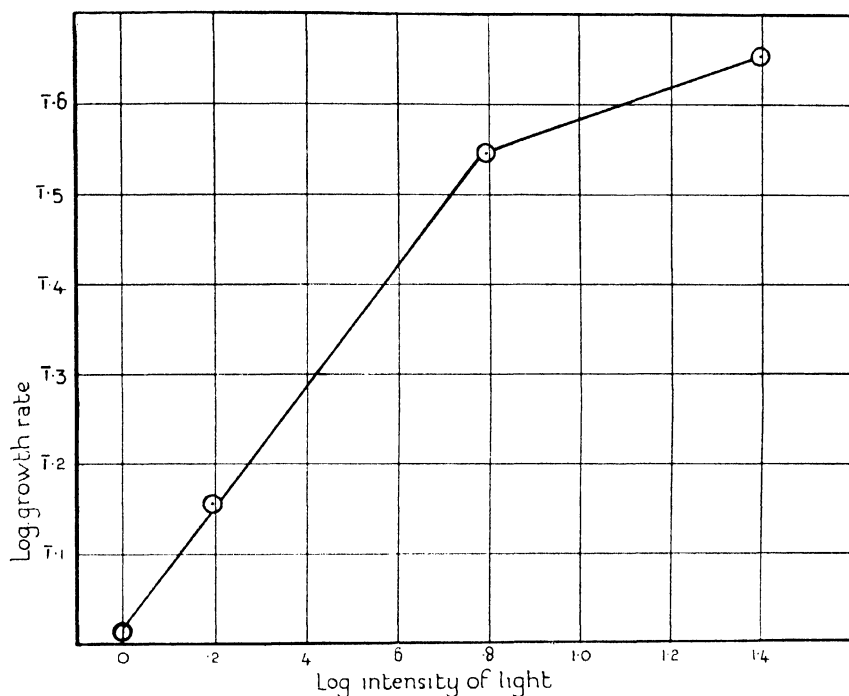


FIG. 6. Diagram showing that with light of low and moderate intensity, the rate of increase in growth rate is approximately proportional to the rate of increase in light intensity. For explanation see text.

ness; while Cultures A, B, and C were placed at distances 16 cm., 32 cm., and 48 cm. respectively from the light, and were therefore illuminated at relative intensities of  $L$ ,  $L/4$ , and  $L/9$  approximately. The adjustment of the thermo-regulator was accidentally altered slightly on the first day, resulting in a rise in temperature of the bath of  $0.5^{\circ}\text{C}$ . for the whole experiment. Culture D unfortunately became contaminated with a fungal mycelium which was first observed on day 5, and by the rapid growth of its entangling hyphae soon made the counting of a representative sample quite impossible. Up to the date of its appearance, however, the values for the bulk of algal protoplasm in the culture lie in a reasonably straight

line, and these values have been accepted as giving roughly the rate of growth of the organism in the dark for this experiment.

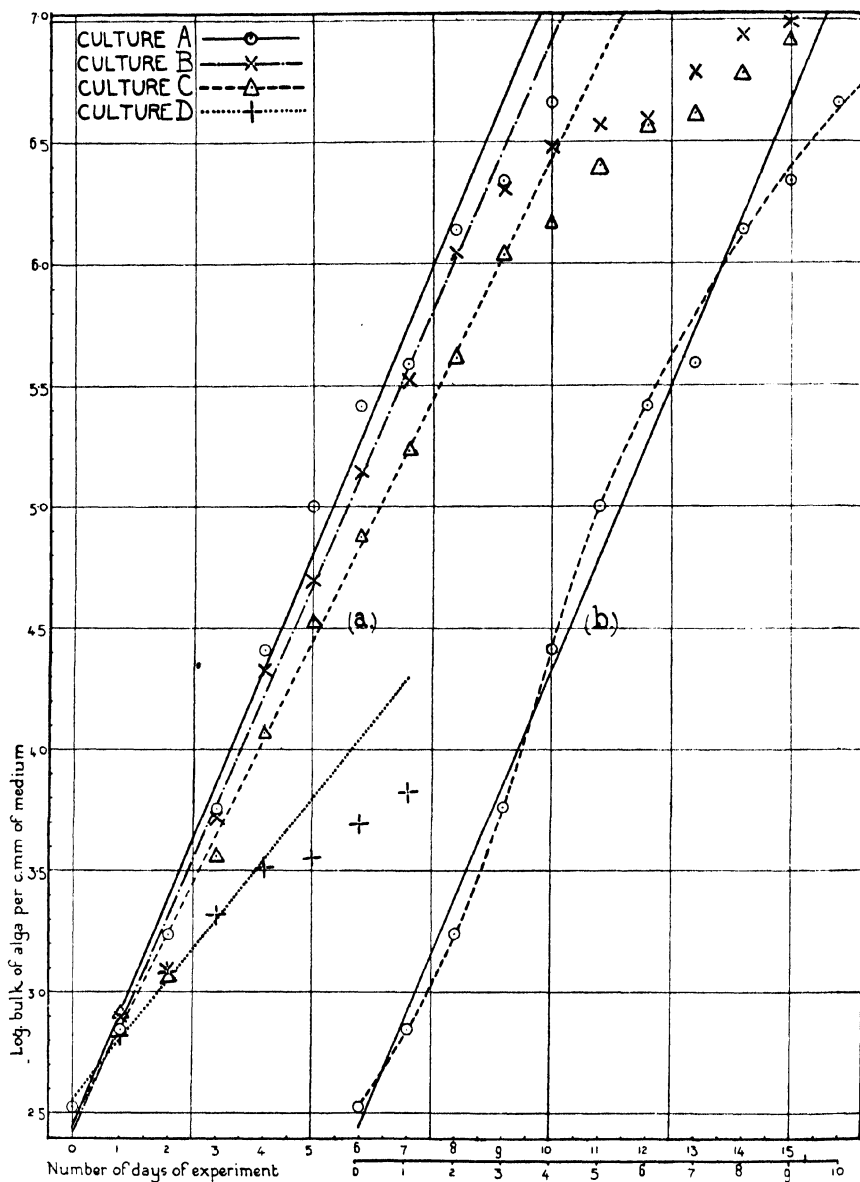


FIG. 7. (a) Diagram showing rates of growth of *Scenedesmus* sp. in a medium containing glucose under illumination of different intensities (Experiment IV). Culture A, intensity  $L$ ; Culture B, intensity  $L/4$ ; Culture C, intensity  $L/9$ ; Culture D in complete darkness in copper-covered flask. (b) Curve for Culture A transferred to separate origin. For explanation see text.

Experimental results are summarized in Table V and plotted in Fig. 7, in which the values for Culture A have been plotted twice, (a) with the first point at the origin common to all four cultures, and (b), for the sake of clearness, with its first point at an independent origin.

The straight lines of nearest fit to the experimental values are expressed algebraically by the following equations:

- (i) Culture A.  $y = 0.471x + 2.44$  with an illumination of intensity  $L$ , and an average growth rate of 0.471, or 100 per cent.
- (ii) Culture B.  $y = 0.45x + 2.42$  with an illumination of intensity  $L/4$ , and an average growth rate of 0.45, or 95.5 per cent.
- (iii) Culture C.  $y = 0.398x + 2.45$  with an illumination of intensity  $L/9$ , and a growth rate of 0.398, or 84.5 per cent.
- (iv) Culture D.  $y = 0.247x + 2.556$  in complete darkness, with an initial growth rate of 0.247, or 52 per cent.

TABLE V.

*Bulk of Algal Protoplasm per c.mm. of Medium (log. values) in Aerated Cultures containing Mineral Salts + 1 per cent. Glucose, under Different Intensities of Light.*

Day of Experiment.	Culture A. Intensity $L$ .	Culture B. Intensity $L/4$ .	Culture C. Intensity $L/9$ .	Culture D. Complete Darkness.
0	2.5250	2.5250	2.5250	2.5250
1	2.8451	2.8954	2.9079	2.8195
2	3.2375	3.0902	3.0783	3.0734
3	3.7545	3.7192	3.5606	3.3187
4	4.4053	4.3209	4.0637	3.5116
5	4.9990	4.6932	4.5271	3.5495
6	5.4152	5.1422	4.8731	3.6895
7	5.5862	5.5220	5.2292	3.8193
8	6.1358	6.0429	5.6117	(too badly contaminated to count)
9	6.3383	6.3036	6.0351	
10	6.6600	6.4742	6.1697	
11	(flask broken)	6.5657	6.4004	
12		6.5922	6.5625	
13		6.7809	6.6153	
14		6.9325	6.7819	
15		6.9892	6.9254	

The relatively small differences between the rates of growth of the alga in the three illuminated cultures, as compared with corresponding cultures in a medium containing mineral salts alone, is a very striking feature of the present experiment. In fact it is very doubtful, since the deviation of the observed points from the straight line calculated for Culture A is rather high, whether the difference in average growth rate between Cultures A and B is really significant; but since similar small differences have been obtained on several occasions, and the values for Culture B are consistently a little lower than those for Culture A, it may be assumed for the sake of argument that the small difference is a real one.

Now supposing that the alga can grow saprophytically at the expense of the readily available glucose at a definite rate depending on the concentration of the glucose in the solution, then Culture D, in which the organism was solely dependent on this source of carbon, will give a measure of the rate of growth possible under these purely saprophytic conditions. And assuming that all the cultures could grow by direct absorption of glucose at the same rate, irrespective of their illumination, then the rates of growth in Cultures A, B, and C due to photosynthesis will be roughly the differences between their total growth rates and that of Culture D, viz. 0.224, 0.203, and 0.151 respectively. That is to say, the alga in Culture B must have been carrying on photosynthesis at nearly the same rate as in Culture A, even though it was receiving light of only one quarter the intensity of that supplied to Culture A.

But the results (not cited in detail) of an independent Experiment IV *b*, identical with Experiment IV except for the absence of glucose from the medium, gave values for the growth rates due to photosynthesis in flasks in the same positions respectively of 0.363, 0.206, and 0.140. It is a striking fact that the values obtained for the rates of growth due to photosynthesis in Cultures B and C under the two sets of conditions are approximately the same in the two experiments, i.e. when calculated directly (Experiment IV *b*) or by difference (Experiment IV); this suggests that with the intensity of illumination in these two positions the two processes of photosynthesis and the direct assimilation of glucose are indeed simply additive, as was indicated by the results of Experiment I. The values for the Cultures A, on the other hand, are very different in the two experiments, showing a potential photosynthetic growth rate of 0.363 when determined by direct measurement in Experiment IV *b* as against a value of only 0.224 when calculated by difference in Experiment IV; this indicates a direct interference between the two processes in the glucose medium with higher light intensities. Moreover, as already shown in Experiment III, a still further increase in illumination to nearly four times the intensity (Culture A, about 9 cm. away from the light) has the effect of increasing the growth rate due to photosynthesis alone to the still higher value of 0.459, whereas, as will be seen in Experiment V, a similar increase in light intensity has very little effect on the rate of growth of the alga in a glucose medium.

The results so far discussed show that under the experimental conditions investigated, the alga can grow at different rates according to the conditions imposed on it, but that there is a maximum value for the growth rate of about 0.475, beyond which the alga has not been observed to go. Under favourable conditions of light intensity this maximum growth rate can be almost attained as a result of photosynthesis alone, showing that the alga is capable of being an entirely self-supporting organism, and under such conditions the addition of directly available glucose to the culture medium

has very little effect in increasing the rate of growth. Under conditions of light less favourable to photosynthesis, the alga apparently is able to make use of directly available glucose in increasing amounts as the light intensity decreases, until it reaches a maximum rate of absorption equal to that which takes place in complete darkness. But the rate of growth due to the direct absorption of glucose has never been observed to be more than half the rate due to photosynthesis under favourable conditions of illumination; so that it is reasonable to infer that in this species photosynthesis is the more normal mode of nutrition and that the saprophytic habit, as exemplified by the direct absorption of glucose, is a secondary process which comes into play under less favourable conditions.

The experimental values for Culture A in this experiment have been plotted apart from those of the other cultures (Fig. 7, *b*) in order to demonstrate an effect which has been observed on a number of occasions in cultures grown under fairly high intensities of illumination. Statistical analyses of the curves derived from these experiments have not yet been fully made, but there is an indication that varying the intensity of the light induces changes not only in the slope of the logarithmic growth curve, but also in its shape; for whereas under illumination of moderate intensity the logarithmic values indubitably lie approximately along a straight line, yet slightly sigmoid curves of the type drawn through the values for Culture A (Fig. 7, *b*) are not uncommon for cultures grown under higher light intensities, and in these cases the straight line of nearest fit can be regarded as representing only the average rate of growth over the period examined. It has already been pointed out that when the light intensity is low, periodic fluctuations may be observed in the logarithmic growth curve.

The inferences deduced from the experiments described above have been drawn from a number of separate investigations which gave results that, though not identical owing to slightly varying external conditions, were sufficiently alike to justify their being used for comparative purposes. It was felt, however, that completely reliable evidence could only be obtained by observing in a single experiment the effects of different light intensities on algal material from the same source both in the presence and in the absence of glucose, as described in the next experiment.

#### (4) *Experiment V.*

The growth of four cultures was investigated, two in a medium containing mineral salts alone (B and D) and two in a medium containing in addition 1 per cent. glucose (A and C). Cultures A and B were placed one on each side of the electric bulb (E) at a radial distance of four inches from the centre of the bulb, with an illumination of intensity 16 l; Cultures C and D were placed side by side in front of the bulb at equal radial distances

of sixteen inches under a light intensity  $l$ , while reflections of light from the surface of the water were prevented by means of a single straight transverse barrier as shown in Fig. 8, O. In this way a measure was obtained of the effect of increasing the light intensity about sixteen times (a) in a mineral salts solution alone, by comparing Cultures A and C, and (b) in the presence of glucose, by comparing Cultures B and D, and also of the effect of adding 1 per cent. glucose to the medium in two different light intensities, by comparing Culture A with Culture B, and C with D.

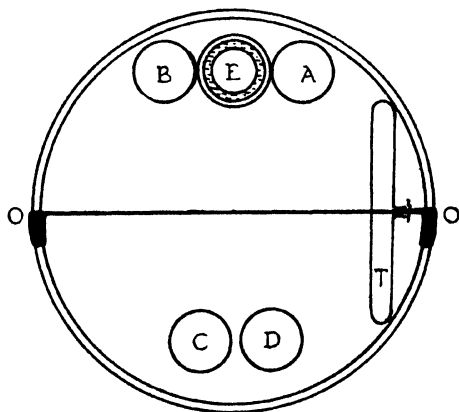


FIG. 8. Ground-plan of the water-bath to show disposition of cultures in Experiment V. E, source of light; A and B, culture flasks at distance 4 inches, and C and D culture flasks at distance 16 inches from centre of E; B and D containing mineral salts only, A and C 1 per cent. glucose in addition; O, barrier to prevent surface reflections; T, bulb of thermo-regulator.

The experimental data are summarized in Table VI and plotted in Fig. 9, the straight lines of nearest fit to the observed logarithmic values being expressed by the following equations:

- (i) Culture A.  $y = 0.473x + 2.518$ , with 1 per cent. glucose under light intensity  $16l$ , growth rate being  $0.473$ , or 100 per cent.
- (ii) Culture B.  $y = 0.462x + 2.496$ , in mineral salts alone under light intensity  $16l$ , growth rate being  $0.462$ , i. e. 98 per cent. approx. of A.
- (iii) Culture C.  $y = 0.377x + 2.563$ , with 1 per cent. glucose under light intensity  $l$ , growth rate being  $0.377$ , i. e. 80 per cent. approx. of A.
- (iv) Culture D.  $y = 0.151x + 2.614$ , in mineral salts alone under light intensity  $l$ , growth rate being  $0.151$ , i. e. 32 per cent. approx. of A, or 40 per cent. of C.

During the whole course of the experiment, Cultures A and B gave astonishingly similar data, both in regard to the number of cells in the medium and to the size of the cells at the time of multiplication, and it is

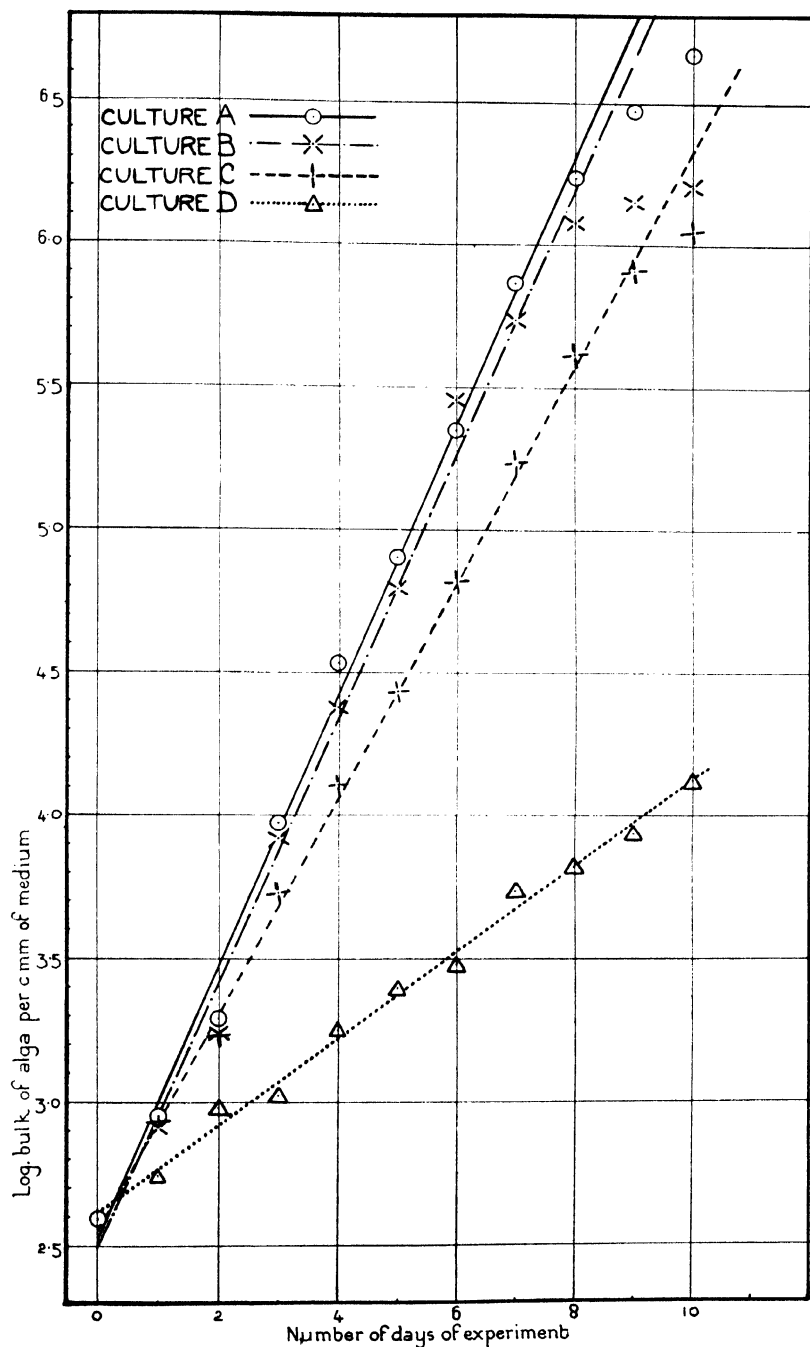


FIG. 9. Diagram showing rates of growth of *Scenedesmus* sp. (1) in mineral salts medium (Cultures B and D) at two different light intensities, 1 (Culture D) and 16 l (Culture B), and (2) in glucose medium (Cultures A and C) at intensities 1 (Culture C) and 16 l (Culture A). For explanation see text.

to be inferred from this that the two cultures were growing as a result of the same physiological process, viz. photosynthesis; for it has frequently been observed that in cultures in which the alga is partly or wholly dependent on the direct absorption of glucose the multiplication of the individual cells is often delayed until they have attained rather larger dimensions than is usual in a purely mineral salts medium. A statistical analysis of the data for these cultures has been carried out, and it is interesting to find that the divergence between the straight lines of nearest fit to the two sets of observations, i.e. the difference in rate of growth of the two cultures (0.011), is less than one-fifth of the standard error of the observations themselves (0.0613) and that the difference in rate of growth of the two cultures is therefore not significant; hence, with an illumination of this intensity the addition of glucose to the medium has no power to increase the growth rate of the organism, which has apparently attained its optimum rate solely by photosynthesis. With Cultures C and D, on the other hand, the position is quite different, for apart from the fact that the dividing cells in Culture C showed an increase in size above the normal, the growth rate is more than double that of Culture D (0.377 to 0.151), showing that with light of this low intensity the saprophytic type of nutrition by direct absorption of glucose is dominant in Culture C.

TABLE VI.

*Bulk of Algal Protoplasm per c.mm. of Medium (log. values) in Media containing Mineral Salts alone and with 1 per cent. Glucose, under two Different Light Intensities.*

<i>Day of Experiment.</i>	<i>Light Intensity 16 l.</i>		<i>Light Intensity 1.</i>	
	<i>Culture A. Min. Salts + Glucose.</i>	<i>Culture B. Min. Salts alone.</i>	<i>Culture C. Min. Salts + Glucose.</i>	<i>Culture D. Min. Salts alone.</i>
0	2.5955	2.5955	2.5955	2.5955
1	2.9499	2.9101	2.9284	2.7404
2	3.2900	3.2314	3.2287	2.9782
3	3.9720	3.9190	3.7264	3.0207
4	4.5281	4.3783	4.1041	3.2472
5	4.9020	4.7922	4.4322	3.3921
6	5.3475	5.4532	4.8196	3.4764
7	5.8641	5.7359	5.2370	3.7331
8	6.2377	6.0780	5.6161	3.8208
9	6.4723	6.1450	5.9062	3.9347
10	6.6700	6.2044	6.0470	4.1225

Further, it is interesting to note that even in the presence of readily available glucose the alga is not able to reach its maximum rate of growth when the light intensity is deficient, but that the increase in rate of growth due to direct absorption of glucose (0.226) is only a little higher than the average rate for all cultures grown in glucose in the dark (0.207); this supports the conclusion drawn from the previous experiments that the saprophytic habit is of secondary importance in the life of this soil alga.

## IV. GENERAL CONCLUSIONS AND SUMMARY.

From the foregoing experiments certain facts emerge which may be briefly summarized.

The alga under observation, *Scenedesmus costulatus*, Chod., var. *chlorelloides*, Bristol Roach, grows in liquid cultures at different rates according to the external conditions imposed upon it, and there appears to be some internal factor which limits the growth of the organism at a temperature of  $24.5^{\circ}\text{C}$ . to a maximum rate in the light represented by the figure  $0.47-0.475$ . This rate of growth has been realized under purely photosynthetic conditions with a comparatively strong light intensity, and under these same conditions the addition of glucose to the medium produces no significant increase in the rate of growth. In fact, the appearance of the cells in these cultures suggests that even in the presence of glucose the alga is purely photosynthetic in nutrition.

As the light intensity diminishes from this value, and the rate of growth by means of photosynthesis becomes less, the alga absorbs glucose directly from the medium to supply the deficiency due to retarded photosynthesis, but only in such quantity as will bring the total growth rate up to the maximum figure,  $0.47-0.475$ . As the intensity of the light continues to diminish, the alga absorbs increasing amounts of glucose up to a maximum quantity, sufficient to produce a growth rate of about  $0.21-0.24$  (?), which is approximately equivalent to the amount assimilable when the alga is grown in complete darkness with glucose as its sole source of carbon.

With light of low intensity, when the rate of growth due to photosynthesis is low, the total rate of growth of the alga possible in a glucose medium is equal to the sum of the rates due to photosynthesis alone and to the maximum amount of glucose assimilable in the dark; increasing the light intensity has no power to increase the amount of glucose absorbed directly; rather it tends, on arriving at a certain value, to check the direct absorption of glucose in favour of autotrophic nutrition.

With low and moderate intensity of illumination, the rate of increase of the growth rate in a mineral salts medium due to photosynthesis alone appears to be directly proportional to the rate of increase in light intensity, until a certain illumination of optimum efficiency is reached; beyond this optimum successive increases in light intensity produce progressively smaller increments in growth rate as the alga approaches more nearly to its maximum rate of growth. The relationship which appears to hold between rate of growth and intensity of light is not sufficiently simple, however, for the determination of its character on the basis of the available data.

Further, the data suggest that the shape of the logarithmic growth curve is subject to certain variations according to the intensity of the light under which the alga is grown. In light of moderate intensity the observed

values lie, within the limits of experimental error, upon a straight line from the time of inoculation for a period of about ten days; in light of low intensity when glucose is absent, and in complete darkness when glucose is present, the curve shows rhythmic fluctuations and the straight line can be regarded only as an average estimate of the changes taking place in the culture; in light of high intensity the curve exhibits a tendency towards a somewhat sigmoid form with a slight lag at the beginning.

In view of the normal habitat of this alga, namely the soil, the above results have a direct bearing on its mode of life in the field and on its influence on soil fertility. When occurring in the lower layers of the soil the organism evidently grows at the expense of certain organic compounds directly available, the evidence from other experiments showing that it is able to use a wide series of substances in the dark (2), and even to secrete an enzyme capable of slowly liquefying gelatine when this is its sole source of food. Consequently, though its proteolytic action may be of direct benefit to other organisms and to the higher plants in breaking down complex substances, it is likely that in its relation to the carbon cycle within the soil the alga should be considered chiefly in the light of its using up some of the energy resources of the soil.

When the alga is growing on the surface of the soil, on the other hand, so long as the moisture conditions are suitable for growth photosynthesis will take place at a rate depending on the intensity of the light; and under favourable conditions the alga will add to the soil very considerable quantities of organic substance with its potential chemical energy. When the light intensity becomes less favourable for photosynthesis, the alga will again begin to absorb available organic compounds from the soil, setting free their chemical energy; but this ability to adopt a partially saprophytic habit will ensure that there is a continual supply of vegetative cells available to resume growth by photosynthesis when the conditions again become suitable.

It must not be assumed that this mode of behaviour towards light and organic compounds is necessarily representative of the condition which holds for all the soil algae, for it has been shown elsewhere (2) that the reactions of the various species that have so far been observed are very different towards a given series of organic compounds; and though *Scenedesmus costulatus* var. is doubtless typical in its behaviour of a large group of species which respond only in a limited degree to the presence of organic compounds, yet there are other species, probably more frequent in occurrence, such as *Chlorella* spp. and *Cystococcus* sp., which respond much more vigorously and in which the saprophytic or semi-saprophytic habit may have become much more firmly established.

Grintzesco (3) affirms that *Chlorella vulgaris* is capable of more vigorous growth in complete darkness than in bright sunlight; Muenscher (4) obtained

approximately equal yields of a large species of *Chlorella*, isolated from soil, in light of moderate intensity and in complete darkness; while the present writer has never yet obtained a culture of *Cystococcus* sp. under purely photosynthetic conditions that has approached to anything like the degree of luxuriance that is invariably attained when certain organic compounds are present in the medium. Such observations, however, cannot be regarded in any sense as critical, and further quantitative experiments with other species are needed, upon lines similar to those described in the present paper, before it will be possible to make a general statement in regard to the relative importance of the photosynthetic and saprophytic habits of the soil algae as a whole.

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#### LITERATURE CITED.

1. BRISTOL ROACH, B. M.: On the Relation of certain Soil Algae to some Soluble Carbon Compounds. *Ann. Bot.*, vol. xl, No. clvii, pp. 149-201, 1926.
2. ———— On the Carbon Nutrition of some Algae isolated from Soil. *Ann. Bot.*, vol. xli, No. clxiii, pp. 509-17.
3. GRINTZESCO, J.: Contribution à l'étude des Protococcacées. *Chlorella vulgaris*, Beyerinck. *Rev. Gén. Bot.*, t. xv, p. 5, 1903.
4. MUENSCHER, W. C.: Protein Synthesis in *Chlorella*. *Bot. Gaz.*, vol. lxxv, p. 249, 1923.



## SOME METHODS OF TECHNIQUE APPLICABLE TO ENTOMOLOGY.

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The following methods of technique, along with many others, applicable to entomological work have been used by the writer in the course of a number of years' experience. Since a considerable proportion of the methods does not appear to be generally known, it is thought that the present article may prove of value particularly to investigators located in parts of the Empire overseas.

*Mounting Media.*

*De Faure's Fluid.*—This is one of the most valuable of all mounting media for microscopical purposes, where small and delicate objects are involved. Its composition, as used by me for a number of years, is as follows:—

Gum arabic	...	...	...	...	...	30 gr.
Chloral hydrate	...	...	...	...	...	50 gr.
Glycerine	...	...	...	...	...	20 cc.
Dist. water	...	...	...	...	...	50 cc.
Chlorhydrate of cocaine	...	...	...	...	...	0.5 gr.

After mixing these ingredients filter.

The medium has the advantage of killing, fixing and mounting in a single operation without the necessity for any previous or subsequent treatment. By virtue of the cocaine present it has the property of fixing whole organisms in an extended condition and is especially valuable for mounting endoparasitic Hymenopterous and Dipterous larvae, Protura, Coccidae and their nymphs, Mymaridae, Thysanoptera, Aleurodidae, certain Aphididae, eggs, etc. In fact almost any organism liable to shrink and distort by other methods, or rendered too transparent in Canada balsam, can, as a general rule, be better displayed in this medium. It is not claimed that uniform success will be achieved in every case, and the user must expect disappointing results in some instances. It is often advisable, but not absolutely necessary, to transfer the larger and less fragile organisms into a small quantity of the medium contained in a watch glass for a few hours before finally transferring to a spot of the fresh fluid on a slide. A few hours after mounting, an object, such as a Dipterous larva, will exhibit its tracheal system with remarkable clarity, showing up even the finer branches with great distinctness. As time goes on, the air gradually disappears from the tracheae, but it is often several months before it departs from the larger trunks. Under English conditions it is not necessary to ring the preparation, and I have examples ten years old that are quite satisfactory; all that is required is to proceed cautiously and lightly when cleaning the cover-glass. As the age of the preparation increases the object becomes more transparent and in some cases very slight distension occurs. Objects such as Dipterous larvae tend to become yellowish brown in colour, but such morphological features as the spiracles, mouth-parts and oral papillae lend themselves to high-power examination. In moist, tropical conditions, where there is relatively little evaporation of the water present in the medium, ringing is desirable either by means of gold size or any other usual method. Experience, however, will soon determine the extent to which this procedure may be necessary.

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*Berlese's Fluid*.—This medium, which was extensively used by the late Prof. Antonio Berlese for the permanent mounting of his great collection of Acarines, has the following composition :—

Distilled water	...	...	...	...	...	20 cc.
Chloral hydrate	...	...	...	...	...	160 gr.
Gum arabic	...	...	...	...	...	15 gr.
Glucose syrup	...	...	...	...	...	10 gr.
Acetic acid	...	...	...	...	...	5 gr.

This medium has very similar properties to de Faure's fluid, in that it kills and fixes an organism in an extended condition. Living specimens may, therefore, be placed directly into the medium on the slide. When desirable they can be killed first by brief immersion in 10 per cent. acetic acid in distilled water or by being placed in boiling water. If, on the other hand, specimens are already preserved in alcohol they should be washed in 10 per cent. acetic acid before transference to the medium. In all cases, after the cover-glass has been put on, the slide should be gently warmed (not allowed to boil) for a short time, then allowed to cool and afterwards left for one or two weeks to dry and set. Finally it is desirable to ring the preparation with gold size or asphalt varnish (obtainable from opticians).

My former colleague, Dr. J. Davidson, who has used this medium extensively for mounting Aphids considers that it is better adapted for the majority of these insects than de Faure's fluid.

*Gilson's Euparal*.\*—This is a mixture of camsal, eucalyptol, paraldehyde and sandarac ; it exists in two forms, the green and the colourless, the latter I consider is to be preferred.

The value of this medium, its superiority to Canada balsam in many cases, and the brevity of the previous treatment required, merit a more general use than is accorded it at the present time. Objects, stained or unstained, after killing and fixation, are passed through graded strengths of ethyl alcohol in the usual way. After transference to 95 per cent. alcohol, they can be placed direct in euparal on the slide and the cover-glass superimposed. The use of absolute alcohol and a clearing agent are thus dispensed with which is a great advantage, especially for workers away from centres of civilisation. The transference from 95 per cent. alcohol to a preparatory agent termed "essence d'euparal" is advocated, but in my experience I have never found this necessary. It is even possible to transfer thin objects, such as preparations of chitinous cuticle, direct from 70 per cent. alcohol to euparal. The latter medium sets hard like Canada balsam but, unlike the latter, does not turn yellow and discolour with age. Preparations made twelve years ago are still as colourless as the slides upon which they are mounted. Its refractive index ( $n=1.483$ ) is lower than that of Canada balsam ( $n=1.524$ ), and for this reason it is also especially valuable for objects which are rendered too transparent by the latter medium. It can be used for all objects which, in the usual way, would be mounted in balsam.

#### *Celluloid Mounting.*

It is incumbent in describing small insects to make microscopical preparations of the male genitalia, mouth-parts, and other structural features. A method which I have used with success is to procure sheets of celluloid, about the thickness of thin Bristol board, or thinner if required for minute, flat objects. Such sheets are cut up into strips of uniform size and slightly beyond the middle a circular hole is made either with a steel punch or a cork-borer.† A set of the latter tools is useful in that it allows of holes of different sizes to be made according to the dimensions of the objects.

\* Obtainable from Messrs. Flatters and Garnett Ltd., 309, Oxford Road, Manchester.

† Suitable punches such as are used for cutting out card discs for mounting small Diptera are obtainable from Messrs. Watkins and Doncaster, 36, Strand, London, W.C.2. Sets of cork-borers can be procured from any dealer in laboratory apparatus.

On the lower side of the hole thus made, a circular cover-glass, of slightly longer diameter, is sealed in position by means of Canada balsam or euparal and allowed to dry. The object is then mounted in any of the media already described and a cover-glass placed over it. An adhesive label is not necessary as the required data can be written on the celluloid with a fine pen and waterproof Indian ink; when dry, permanency can be secured by painting over the written data with the thinnest possible coating of euparal or balsam. In order to ensure neatness it is best to use very fluid xylol balsam and apply it with a fine brush. Finally a pin is inserted through the celluloid below the data as with an ordinary carded specimen.

The advantage of this method is that the object can be pinned in its proper place in an ordinary collection of dried insects. Or, a whole collection of such insects as Anoplura, Aphaniptera, larvae, etc., can be mounted in this manner, each species being placed in its correct taxonomic position in cabinet drawers or store boxes. A collection or an individual object, mounted in this manner, is readily available for microscopical examination; when high powers of the microscope are desired, all that is necessary is to withdraw the pin, and the whole preparation then becomes comparable with an ordinary micro slide. The most convenient size of the celluloid slips is about 2 ins. by 1 in. but larger or smaller dimensions can be adopted of course if desired in conformity with the size of the object.

#### *Staining.*

*Fuchsin.*—In the present article I am only dealing with the staining of those chitinous structures which are too transparent when mounted in an unstained condition to be readily studied with accuracy. Under this category come the wings of many minute parasitic Hymenoptera; the wings of the smaller Diptera, especially those with the venation ill-defined, or with obscurely discernable vestigial veins; the pores and gland openings on the pygidium and other regions of Coccidae; the mouth-parts, spiracles, etc., of minute larvae; in fact any transparent delicate part or organ whose structure is difficult to discern by ordinary procedure. The advantages derived from the proper staining of such structures are evident from the facility with which they can be studied and the consequent relief from undue eye-strain, the accuracy with which they can be drawn and measured, and the elimination of the possibility of the less defined structures being inaccurately interpreted.

The stain most generally used and recommended is fuchsin, and ordinary fuchsin or magenta is a basic dye and is the hydrochloride of rosanilin. Acid fuchsin is the sodium salt of a sulphonic acid derived from fuchsin. Both acid and basic fuchsin are used as chitin stains, but in my experience basic fuchsin gives the better results. In the form known as Ziehl's carbol-fuchsin it is extremely effective and is usually made up by taking 10 cc. of a saturated solution of basic fuchsin in 95 per cent. alcohol and 100 cc. of a 5 per cent. solution of carbolic acid in distilled water. The two are then shaken together and the stain is ready for use. For delicate and refined technique the emended formula given in McClung's Microscopical Technique, 1929, may be used, viz. :—

#### Solution I.

Basic fuchsin (90 per cent. dye content)	...	0.3 grms.
Ethyl alcohol (95 per cent. strength)	...	10 cc.

#### Solution II.

Carbolic acid	...	...	...	...	5 grms.
Distilled water	...	...	...	...	95 cc.

Solutions I and II are then mixed for use.

Material is transferred to the stain and left there as long as may be necessary ; if very rapid staining is required, slightly warming the stain containing the object in a watch-glass will accelerate its action. On the other hand, if an object be left for 14 hours or more in the stain no harm will ensue, and decolorisation to the appropriate degree may be achieved by transference to 95 per cent. alcohol containing 3 per cent. by volume of concentrated hydrochloric acid. It is always desirable to leave the specimens slightly darker stained than is necessary as a certain amount of subsequent decolorisation occurs. If mounting is done in Canada balsam, clearing should be by means of carbol-xylol, and the balsam, it may be added, should be xylol balsam.

A simpler stain is obtained by taking 1 gram. of basic fuchsin (magenta crystals) and dissolving it in 100 cc. of 95 per cent. alcohol, as has been recently advocated by Ferris.\* Any excess of the stain can be removed by washing in 95 per cent. alcohol ; this should be done as quickly as possible under a microscope or too much loss of colour results.

#### *Decolorising Chitin, etc.*

It often happens that chitinous parts are too darkly coloured to admit of the clear definition of structural details. This disadvantage may be overcome by taking a small stoppered phial and covering the bottom with a few drops of concentrated hydrochloric acid diluted by half its bulk of distilled water. Add a few crystals of potassium chlorate and very soon the greenish-yellow fumes of chlorine become evident in the phial. The part to be decolorised should be immersed quickly in the liquid and left there until it becomes paler in colour. After removal and momentarily washing in dilute alcohol it is ready for mounting. Specimens covered with thick black chitin are difficult to bleach, and several days' treatment are usually necessary. Very delicate objects that will not withstand the acid can be suspended on a piece of moist filter paper in the phial so as to come into direct contact with the chlorine vapour only.

In the case of Collembola and other insects in which structural details are often concealed by a heavy deposit of dark hypodermal pigment, decolorisation can be effected by treatment with 10 per cent. potassium hydroxide and afterwards washing in distilled water.

#### *Dissecting Fluids.*

For the dissection and examination of minute delicate objects, including poly-embryonic phases, glands, blood cells, oenocytes, fat-body, parasitic micro-organisms, etc., in the living condition, normal salt solution often causes rupture or distortion of the parts or cells it is desired to examine. The insect is best opened in Ringer's solution whose composition is as follows :—

Sodium chloride	...	...	...	...	0.8	gram.
Calcium chloride	...	...	...	...	0.02	gram.
Potassium chloride	...	...	...	...	0.04	gram.
Sodium bicarbonate	...	...	...	...	0.02	gram.
Distilled water	...	...	...	...	100	cc.

As an alternative, and much quicker to make up, Pictet's liquid, which is a 1 per cent. to 3 per cent. solution of manganese chloride, is recommended by Bolles Lee.†

#### *Preserving Fluids.*

*Alcohol.*—For ordinary purposes ethyl alcohol is in general use. The strength

\* Principles of Systematic Entomology, Stanford Univ. Publications Biol. Sciences, v, no. 3, 1928.

† Microtomist's Vade-Mecum.

commonly adopted is 70 per cent. but in many cases this results in course of time in maceration supervening. I prefer 80 per cent. alcohol to which five parts by volume of glycerine have been added. With larvae and pupae, shrinkage and often distortion result if placed in the alcohol straight away. These disadvantages can be greatly reduced, or almost eliminated, if the specimens are first placed in 40 per cent. alcohol which is gradually warmed up to the boiling point. On allowing to cool down they should be transferred to 70 per cent. alcohol and finally to 80 per cent. strength.

Specimens so treated are usually sufficiently well preserved for subsequent study or descriptive purposes.

*Pampel's Fluid.*—This is useful for keeping material for a length of time with the tissues in a soft flexible condition for subsequent dissection. After chloroforming the insect a small slit is made along the side of the abdomen to allow of ready penetration by the preservative fluid. It was first used by Pampel\* and later by Miss I. Florence† in her studies on the Hog Louse and by other workers. It is particularly useful in cases where, for example, important characters are afforded by the genital organs and where dissection cannot be made straight away. Its composition is as follows :—

Glacial acetic acid	...	...	...	...	4 parts.
Distilled water	...	...	...	...	30 parts.
Formaldehyde	...	...	...	...	6 parts.
95 per cent. alcohol	...	...	...	...	15 parts.

*Lavdowsky's Solution.*—This method is very simple and practical and is best adapted for large objects such as Lepidopterous larvae, where rapid penetration takes place with greater facility than in very small organisms. The method has been slightly modified by Tothill‡ whose recommendations I have found to be of great value. Place the insect, or part of an insect, in 5 per cent. chloral hydrate in distilled water and gently warm for a minute or so until the temperature does not quite reach boiling point. The object is then left on a piece of filter paper for about five minutes, until the fluid has drained away. It is then transferred to a bottle of fresh chloral hydrate of the same strength and is left therein for one week. It is removed and drained a second time, and afterwards submerged in further chloral hydrate. The bottle is then tightly corked, and the specimen will keep for several years in a condition fit for dissection. The complete penetration of the chloral hydrate is ensured by making a slit along one side of the insect with a surgeon's needle or other suitable instrument.

### *Storage of Spirit Material.*

One of the greatest difficulties in the storage of valuable spirit material in small phials is the ultimate shrinkage and deterioration of the corks. This results in evaporation of the alcohol and the necessity for frequent examination of such a collection to avoid the specimens drying up altogether. This disadvantage may be obviated in one of two ways.

The specimens can be transferred to phials of alcohol in the usual way and the mouth of each phial closed either with a piece of fine muslin tied securely round with cotton, or loosely plugged with cotton wool. The phials are then inverted in a straight-sided stoppered museum jar previously filled with alcohol. Specimens thus taken care of remain a long time without attention and all that is necessary is to add

\* Pampel, W. 1914. Die Weiblichen Geschlechtsorgane der Ichneumoniden.—Zeits. für wiss. Zool., 108, pp. 290–357.

† Florence, L. 1921. The Hog Louse, *Haematopinus suis*, Linné.—Mémor. 51, Cornell Univ. Agric. Expt. Station.

‡ Tothill, J. D. 1919. The Chloral Hydrate Method of Preserving Insects for Dissection.—Proc. Nova Scotia Ent. Soc. (I am indebted to Dr. J. W. Munro for this reference.)

occasionally to the alcohol that evaporates in the jars. It is, of course, easy by this arrangement, to keep a number of species of a genus each in its own phial in a single jar.

Or the use of phials may be dispensed with and each specimen enclosed in a short length of glass tubing whose bottom had been previously sealed in a Bunsen burner. The tube is partly filled with 80 per cent. alcohol and the specimen introduced. Nearly all the alcohol is then poured off and the tube drawn out to a point, at some distance from the object, by means of a Bunsen flame. After breaking it off at the neck it is then filled with alcohol by means of a fine tubular funnel. The broken off end is then sealed in the flame and the process finished. Care and practice are required to carry out the operation neatly and it is important to ensure that the broken off neck is as small as is practicable, so as to admit of rapid sealing in a hot flame. A label is best introduced into the tube along with the specimen. I first came across this method in 1905 when examining the Haviland collection of termites at Cambridge which are preserved in sealed up lengths of tubing. The earliest published suggestion I know of is a note by Nachtrier,\* who also recommends equal parts of alcohol, glycerine and water (Fleming's fluid) in place of pure alcohol.

### *Methods of Rearing Insects.*

*Iron Rings as Covering Frames.*—A number of years ago Mr. E. E. Green recommended to me the use of heavy iron rings covered with muslin for closing circular breeding vessels. This very simple device dispenses with the time taken in securing pieces of muslin by means of string or rubber bands each time the receptacles are opened. The rings require to be made slightly larger than the diameter of the receptacles to be used, and since their weight exerts a tension on the muslin, the insects are precluded from escaping. A stock of such rings of different sizes can be readily made by a blacksmith, at small cost, from iron rods of varying diameter (according to the weight needed).† In order to prevent rusting they can be painted with aluminium paint or other preservative before the muslin is sewn on. A quicker method is to cut out circles of muslin of the required diameter and fasten them to the rings with marine glue.

*Petri Dishes.*—For a number of years past I have adopted ordinary double Petri dishes as used by bacteriologists for plating cultures. The advantage of this method is the ease with which they can be kept sterile while the contained insects can be readily observed under a binocular microscope. Various kinds of small insects can be reared in these receptacles, and for studying many of the parasitic Hymenoptera, for example, they are invaluable, because the behaviour of such parasites in relation to their hosts can be clearly watched. A suitable-sized Petri dish is about 9 cm. diameter and 1.5 cm. in height (inside dimensions) for the lower dish, and it should be stipulated when purchasing that the edge of the lower dish is ground perfectly flat so that the inverted upper dish reposes accurately upon it; this precaution is necessary or minute insects would be liable to escape. If desired, the upper or covering dish can be replaced by one of the heavy iron muslin-covered rings already alluded to.

*General Rearing Cage.*—An inexpensive type, which admits both light and air and at the same time allows of free observation, can be quickly put together as follows. A hurricane lamp chimney of as wide a mouth as possible is fitted over an ordinary plant pot of suitable size, and the upper opening of the lamp chimney is closed with one of the muslin-covered rings already mentioned. The plant pot serves to hold a phial containing water to keep the food-plant fresh, and the space between the phial and the pot may be filled with fine soil or sand. Where soil is not needed, as with Hemiptera,

\* Nachtrier, H. F. *Journal Royal Microsc. Soc.*, 1900.

† A very convenient size is 6 ins. outside diameter, made from rod *not less than* 1 in. in circumference.

the lower opening of the lamp chimney can be closed with a cork bung perforated to allow of the stems of the food-plant being placed in the water below. Most kinds of plant-feeding insects can be reared in cages of this type, and the ease with which the latter can be kept clean is greatly in their favour.

*Receptacles for Soil Insects.*—Subterranean insects are often notoriously difficult to rear, owing to the fact that once soil is placed in a pot or other receptacle, physical changes supervene which render it difficult to maintain natural conditions. Wireworms, Melolonthoid larvae, and other true soil insects can be reared successfully if the pots containing the soil are let down into the ground to within about an inch of their rims. The pots must be unglazed and when placed *in situ* the moisture content and temperature are maintained in a condition approaching that of the surrounding soil. The pots require to be suitably covered and the nature of the covering naturally depends upon whether they contain growing plants or not. The opening at the bottom of the pot requires to be enlarged to admit of free drainage and closed with gauze or perforated zinc. If such receptacles are not freely exposed to the weather, or periodical watering is necessary, a very small sized earthen pot, inserted almost flush with the surface of the soil, in the centre of the larger pot, is desirable. This small pot can be filled with a uniform amount of water in the case of critical experiments, but its main use is to prevent the soil from caking at the surface, which almost invariably happens if water be applied in the usual manner. With some insects, such as wireworms, chafer larvae, etc., which at certain times of the year penetrate deeply into the soil, unglazed drain pipes are often better than plant pots. The taller vessels allow of the free migration of the insects through a great vertical distance in the soil.



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# ON THE OCCURRENCE OF THE PARTHENO- GENETIC AND SEXUAL FORMS IN *APHIS RUMICIS* L., WITH SPECIAL REFERENCE TO THE INFLUENCE OF ENVIRONMENTAL FACTORS

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(With 6 Text-figures.)

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## I. INTRODUCTION.

In an earlier paper<sup>(5)</sup> the writer gave a short review of the more recent literature dealing with the occurrence of alate and apterous parthenogenetic females in aphides. Since that time several investigators have brought forward experimental evidence showing the influence of external factors on the occurrence of these forms, notably Brittain (3), Mason (17),

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Wadley<sup>(23)</sup>, Ewing<sup>(11)</sup>, Ackerman<sup>(1)</sup> and Reinhard<sup>(19)</sup>. In most cases these writers have briefly summarised the views of previous observers so that it is not necessary to do so here.

The older views regarding the influence of environmental factors on the occurrence of the sexuales in aphides were also briefly discussed in my earlier paper<sup>(5)</sup>. Little experimental work has been done on this problem, although it is apparent from field observations on several species that external factors play a large part in bringing about the cyclical change in these insects. Since Klodnitzki's (1912) extensive studies, which were dealt with in my previous paper, two further contributions to the literature are of particular interest. Uichanco<sup>(21)</sup> discusses the modifying influence of environmental factors on reproduction in the Aphididae and a later paper<sup>(22)</sup> contains an excellent account of the embryogeny and post-natal development of the Aphididae. Marcovitch<sup>(14,15)</sup> shows the importance of length of day as a factor affecting migration and the occurrence of the sexual forms in aphides.

In the present paper an account is given of the results obtained with *Aphis rumicis* L. (*A. fabae* Scop.) from rearing experiments carried on during the past 7½ years, particularly with reference to the two phenomena of the life-cycle referred to above.

The aims in view were (a) To trace the normal sequence of the generations in the complete life-cycle and the occurrence of the parthenogenetic and sexual phases. (b) To see whether the normal sequence could be affected experimentally by changing the environment in which the aphids were reared. (c) To observe the influence of environmental changes on the occurrence of alatae and apterae.

The experiments were commenced in June 1920 (Line A) with two apterous viviparous females taken from a wild colony on beans found in a local garden, and nine further related parthenogenetic lines were reared during succeeding years.

## II. METHODS OF REARING THE VARIOUS PARTHENOGENETIC LINES.

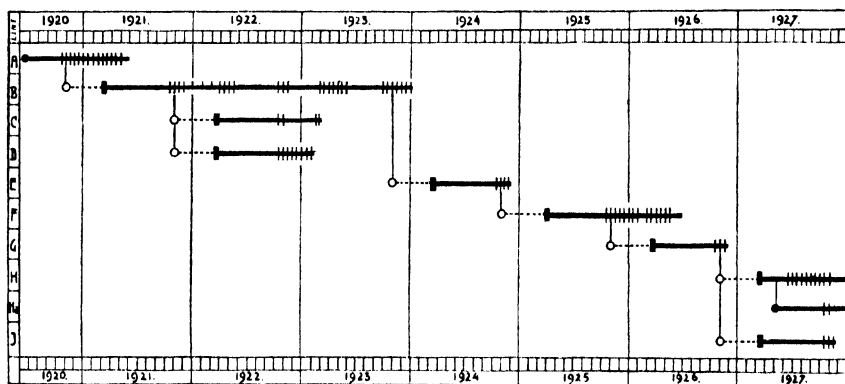
The methods employed in rearing the aphids have been already described<sup>(9)</sup>. The relationships of the ten parthenogenetic lines are shown in Fig. 1 and Table I. In each of the eight lines, started with a Fundatrix, the aphids were reared on *Euonymus europaeus* until alate migrants developed (usually in 3rd generation), and afterwards on Longpod beans until the line was discontinued. In each year, about the end of August, colonies from the beans were established on *Euonymus* so as to ensure that sexuales would be obtained and fertilised eggs laid. When the

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Table I.

*Showing relationships of the 10 parthenogenetic lines of A. rumicis.*

Line	Started with	Taken from line	Parthenogenetic generations		Ova	
			Started	Ended	Laid	Com- menced to hatch
A	2 apterae	Wild colony	16. vi. 20	30. v. 21	Oct.-Nov. 1920	8. iii. 21
B	Fundatrices	A	8. iii. 21	31. xii. 23	Oct.-Nov. 1921	23. iii. 22
C	"	B	23. iii. 22	28. ii. 23	" 1922	None hatched
D	"	B	23. iii. 22	8. ii. 23	" 1923	17. iii. 24
E	"	B	17. iii. 24	30. xi. 24	Transfers not made for this purpose	
F	"	E	7. iv. 25	21. vi. 26		
G	"	F	24. iii. 26	30. xi. 26	Oct.-Nov. 1924	7. iv. 25
H	"	G	15. iii. 27	31. xii. 27	" 1925	24. iii. 26
Ha	Apterae	H	6. v. 27	31. xii. 27	" 1926	15. iii. 27
I	Fundatrices	G	20. iii. 27	9. xi. 27	" 1927	—

Fig. 1. Chart showing the relationships of the ten parthenogenetic lines of *Aphis rumicis* used in the experiments.

The years are divided into monthly periods. The broad black bands show the periods during which continuous parthenogenetic reproduction was maintained in each parthenogenetic line: the short cross-lines indicate the periods during which sexual forms occurred in the colonies: where the cross-lines are absent, only parthenogenetic individuals were obtained. Line B was started from one fertilised egg taken from line A and lines C-I are of the same strain, being related as shown with the thin connecting vertical lines.

With the exception of lines A and Ha each one was started from a fertilised egg.

○ = fertilised ovum.

■ = fundatrix.

● = apterous viviparous female.

During the winter period the aphids were reared under varying temperatures and with line H they received only 8 hours daylight daily from March to November. See also Figs. 4 and 5.

fundatrices began to hatch out the following spring, new lines were started as shown in Fig. 1.

Line A was started from a wild colony. Line B was started with one fundatrix from line A and since the subsequent lines were descended from line B, a single strain of the species has been used throughout. The aphids were reared in a large open glasshouse during the summer period, or in the open air insectary and in a heated glasshouse during the winter period. The air temperature at Rothamsted during the  $7\frac{1}{2}$  years is shown in Figs. 3 and 4 and also the temperature during the winter period in the heated glasshouse. The temperature in the summer glasshouse (1920-21) and open-air insectary (1927), during the summer period, is also shown. It will be seen that the temperature in the insectary approximates closely to that of the outside air temperature plotted from Rothamsted records and the temperature in the glasshouse during the summer period shows an average mean about  $10^{\circ}$  F. higher than the outside air temperature.

The *Euonymus* plants on which ova were laid in autumn, were kept outside in the open air during winter, being removed to the summer glasshouse in spring, soon after the fundatrices commenced to hatch out. By reference to Figs. 3 and 4 the conditions under which the aphids were reared can be seen from the following data: the symbols *O* = open air; *O.G.* = summer glasshouse; *H.G.* = heated winter glasshouse. It will be noted that the dates given for aphids in the open air (*O*) refer to living aphids; the ova from which the fundatrices were obtained were kept outside during winter.

*Line A.* 16. vi to 12. x. 20 (*O.G.*); 13. x. 20 to 31. iii. 21 (*H.G.*), 1. iv to 30. v. 21 (*O.G.*). *Line B.* 8. iii to 31. iii. 21 (*O*); 1. iv to 9. x. 21 (*O.G.*); 10. x. 21 to 20. iii. 22 (*H.G.*); 21. iii to 31. x. 22 (*O.G.*); 1. xi. 22 to 28. iii. 23 (*H.G.*); 29. iii to 19. x. 23 (*O.G.*); 20. x to 31. xii. 23 (*H.G.*). During the period 12. xi. 22 to 12. i. 23 the colonies received artificial light from electric lamps in addition to normal daylight (see Davidson, *Journ. Sci.* 1924, LIX, p. 364). A control series under the same temperatures received only normal daylight. *Line C.* 23. iii to 31. x. 22 (*O.G.*); 1. xi. 22 to 28. ii. 23 (*H.G.*); as in line B the colonies received artificial light, control colonies receiving only normal daylight. *Line D.* 23. iii to 31. x. 22 (*O.G.*); 1. xi. 22 to 8. ii. 23 (*H.G.*); the temperatures for this line, during the winter period, were lower than for B and C as can be seen in Fig. 3 (1927, middle line). *Line E.* 17. iii. to 30. xi. 24 (*O.G.*). *Line F.* 7. iv to 19. iv. 25 (*O*); 20. iv to 8. xi. 25 (*O.G.*); 9. xi. 25 to 20. iv. 26 (*H.G.*); 20. iv to 21. vi. 26 (*O.G.*). *Line G.* 24. iii to 30. xi. 26 (*O.G.*). *Line H.* 15. iii to 20. iv. 27 (*O.G.*); 21. iv to 30. ix. 27 (open-air insectary); 1. x to

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31. xii. 27 (*H.G.*). From 28. iii to 5. ix. 27 the colonies in this line received only 8 hours daylight daily, being placed in a dark box from 5.30 p.m. until 9.30 a.m. daily. From 25. x to 23. xii. 27 the colonies were submitted to artificial light from electric lamps from sunset until 10 p.m. during 5 days each week: a control series reared under the same temperatures received only normal daylight, being kept in a dark box during the illumination period. *Line Ha.* 15. iii to 20. iv. 27 (*O.G.*); 21. iv to 30. ix. 27 (open-air insectary); 1. x to 31. xii. 27 (*H.G.*): this was a control line for line H, and after 5. v. 27 the aphids received normal daylight but, as in line H, a series of colonies received artificial light during the period stated and a control series had only normal daylight. *Line I.* 20. iii to 20. iv. 27 (*O.G.*); 21. iv to 9. xi. 27 (open-air insectary): this was a further control line for line H and the aphids received normal daylight.

### III. NORMAL OCCURRENCE OF THE PARTHENOGENETIC AND SEXUAL FORMS.

The normal life-cycle of the bean aphid as it occurs in England is shown graphically in the following diagram, together with the terms used in the present paper. A reference to this diagram will enable the reader to follow more readily the details discussed later.

### IV. NORMAL OCCURRENCE OF PARTHENOGENETIC ALATAE AND APTERAE.

It is clear from Fig. 2, that, if the normal bi-sexual cycle is to be completed, alatae must develop during two critical periods of the cycle, namely in spring when migration takes place from the winter host to the summer food-plants and again in autumn when the alate sexuparae (re-migrants) are due to return to the winter host plant. Furthermore, during the summer period, owing to the rapid reproduction of this species, it is necessary that alatae develop from time to time so as to prevent the starvation of the aphids in overcrowded colonies, and to ensure the distribution of the species to other food-plants. Since the sexual females (apterous) are produced by the alate sexuparae, it follows that, if experimental conditions are established such that a continuous line of apterous parthenogenetic females only are produced, the normal bi-sexual cycle cannot be completed. The occurrence of alatae therefore is closely associated with these three important features of the life-history and the environmental factors which influence migration and the change from the parthenogenetic to the sexual method of reproduction must also be considered in this respect as exercising an influence on the occurrence of

apterae and alatae. The alate form is the primitive condition and it seems to the writer, as already stated in a recent paper (10), that the logical interpretation of the problem of the occurrence of the alatae and apterous parthenogenetic females is, What factor or factors make for the occurrence of apterae? since by the continuation of a purely apterous parthenogenetic line, the distribution of the species is severely limited and the completion of the normal bi-sexual cycle is prevented.

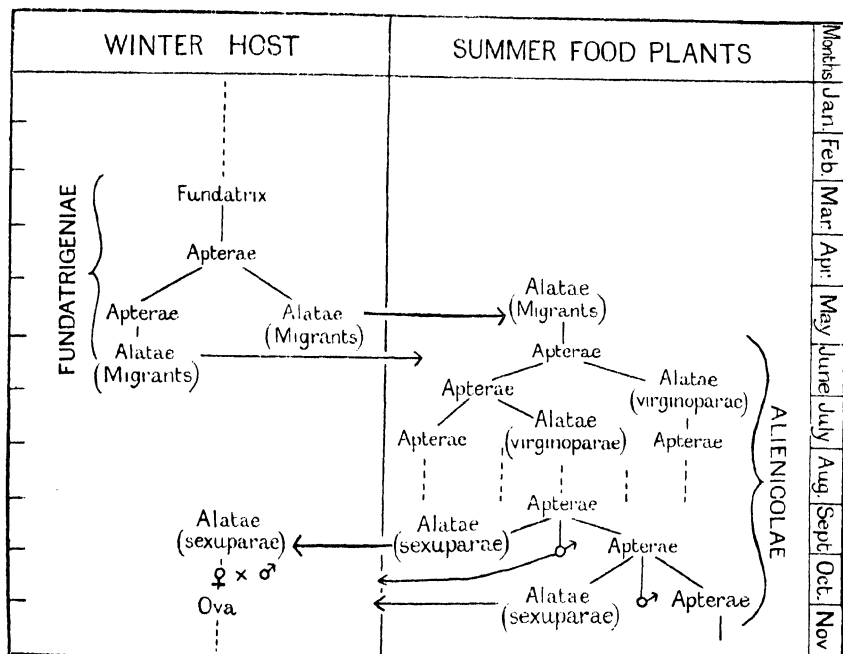


Fig. 2. Diagram illustrating the life-cycle of *Aphis rumicis*, showing the terms used for the different forms in the various generations.

### V. THE FUNDATRIGENIAE GENERATIONS.

The fundatrigeniae are the descendants of the fundatrix, born on the winter host (Fig. 2) and consist of apterae and alatae, the latter being the migrants.

#### (a) Offspring of the fundatrices.

The offspring of the fundatrices are usually apterous but may consist of a mixed brood of apterae and alatae, the former being usually in the majority. The proportion of alatae which develop is affected by the condition of the food-plant (nutrition factor) and by the amount of young growth available in relation to the size of the colonies (overcrowding).

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Counts made of the individuals present in four separate colonies on *Euonymus* produced by four fundatrices, after periods varying from 12 to 19 days, gave the following percentages of apterae—100, 83, 97, 11.5. The latter plant was “woody” and not making young growth, whereas on the other three plants there was plenty of young growth. Other instances were observed in which fundatrices produced a majority of alatae on “woody” *Euonymus* plants. When young shoots were maintained by cutting back the plants and overcrowding avoided, apterous fundatrigeniae produced a larger proportion of apterae in several successive generations. Fundatrices reared on Longpod beans and on *Rumex* produced freely and the offspring developed chiefly into apterae.

## (b) *Offspring of the fundatrigeniae apterae.*

The offspring of the apterae of 2nd generation on *Euonymus* develop chiefly into alatae (3rd generation) and although a few apterae may occur in this 3rd generation their offspring develop into alatae, so that after about three generations the spindle tree becomes free from the aphid, owing to this tendency for alate migrants only to develop. This sequence under natural conditions is correlated with overcrowding and nutrition, and the condition of the spindle tree does not favour the development of apterae when the flush of early spring growth is over. There is, however, apparently an inherent tendency for alate migrants to develop in these early generations on *Euonymus*, which is evidently associated with the evolution of the migrating habit. This tendency is affected by overcrowding and nutrition. Consequently, the proportion of alatae and apterae in a colony may vary considerably according to these conditions.

Table II.

*Showing offspring of fundatrigeniae apterae reared on Euonymus (1-7) and beans (8, 9).*

Colony no.	Date apterae transferred	No. transferred	Alatae and apterae in colony after 10 days		
			Days	Apterae	Alatae
1	25. iv. 14	2	25	0	Many
2	5. iv. 21	5	21	Few	„
3	5. iv. 21	6	21	„	„
4	30. iv. 22	1	19	0	58
5	25. iv. 22	1	21	1	44
6	30. iv. 22	1	19	1	55
7	9. v. 27	9	19	20	400
8	6. v. 27	2	18	19	2
9	6. v. 27	6	18	71	27

If they are favourable, apterae may be obtained in several successive generations.

In Table II (Nos. 1-7) examples are given showing the strong tendency for the offspring of apterae to develop into alatae when reared on *Euonymus*.

When reared on beans a higher proportion of apterae developed as shown in Nos. 8 and 9. Similarly on *Rumex* the proportion of apterae was greater. This appears to be due to the better nutrition afforded by these plants.

(c) *Offspring of the fundatrigeniae alatae (migrantes).*

The offspring of the migrantes are normally laid on intermediate food-plants and develop into apterae. When reared on *Euonymus*, on which plant they reluctantly reproduce, apterae develop if suitable young growth is available, but if the plant is "woody," alatae will also develop, which is further evidence of the unsuitability of *Euonymus* as a permanent food-plant and of the effect of nutrition on the occurrence of alatae. When successive generations, from the fundatrix, are reared on *Euonymus*, alatae tend to predominate (*vide* (5), p. 305)<sup>1</sup>.

## VI. THE ALIENICOLAE GENERATIONS.

The alienicolae generations are initiated by the alate migrants and we have seen that the unsuitability of the spindle tree as a summer food-plant and overcrowding are important factors affecting the progress of the rhythmical spring migration. The descendants of the offspring of alate migrants can be considered as alienicolae so long as an unbroken line of parthenogenetic generations is maintained. In line B, for instance, a line of alienicolae was carried on for  $2\frac{1}{2}$  years.

The following forms may develop in the alienicolae generations: (a) apterous viviparous females; (b) alate viviparous females (virginoparae) which produce parthenogenetic viviparous females; (c) alate viviparous females (sexuparae) which produce apterous sexual females; (d) alate males. The occurrence of these various forms is influenced by environmental factors, particularly length of day, overcrowding, nutrition and temperature. In nature, continuation of the parthenogenetic

<sup>1</sup> It is of interest to note in this respect that the autumn re-migrants (sexuparae) lay the sexual females below the old leaves of the spindle tree and the latter feed along the midrib and secondary veins, until they go to the branches in order to lay their eggs. The sexual females, therefore, are not dependent upon young growth. On the other hand, the spring migrants and also the alate virginoparae of the summer generations lay their offspring on or near the young growth of plants, on which the latter instinctively feed.

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generations over the winter period is limited by seasonal and climatic factors. Under experimental conditions, however, favourable temperature and nutrition may be maintained and parthenogenetic reproduction carried on for long periods. Even under these conditions, the progress of the colonies during the winter period is slow compared with the summer period indicating the importance of the light factor<sup>1</sup>.

### A. OCCURRENCE OF ALATAE AND APTERAE.

As referred to above, the alatae may be virginoparae or sexuparae in addition to males. Actually in nature, in England, the two latter forms do not occur until late September, or early October, a period coincident with a falling mean temperature, decreasing hours of daylight and scarcity of suitable food-plants (poor nutrition). The alate virginoparae which develop during the summer period are to be considered as dispersal forms, whose function is to ensure the distribution of the species. Under experimental conditions these forms may develop during the winter period, as will be described later, although, with moderate temperatures the alatae produced during that period tend to be sexuparae. The occurrence of alate sexuparae in autumn marks the appearance of the sexual phase, and the environmental factors concerned, as we shall see later, are correlated with rhythmical seasonal changes. These factors are (a) length of day, (b) temperature, and (c) plant growth (nutrition) as affected by (a) and (b).

#### (1) *Offspring of the alienicolae apterae.*

The offspring of the apterae may develop entirely into apterae or alatae or consist of a mixed brood of apterae and alatae. The alatae may consist of virginoparae, sexuparae and males. The two latter occur when the sexual phase is in evidence and all the alatae at this time may be

<sup>1</sup> Several species have been reared over long periods in a continuous parthenogenetic line.

(1) Slingerland (1893), according to Uichanco (1921), reared *M. persicae* for 62 generations over a period of 2 years 10 months.

(2) Ewing (1916) reared *A. avenae* for 87 consecutive parthenogenetic generations in California.

(3) Paddock (1919) reared *A. gossypii* for 51 generations.

(4) Comstock (*Introduction to Entomology*, 1924, p. 417) states that Slingerland carried on a species for 98 generations over a period of 4 years and 3 months.

(5) Reinhard (1927) reared *A. gossypii* for 59 generations in Texas.

(6) The writer reared *A. rumicis* through 50 generations (line B) in a period of 2 years and 10 months at Rothamsted. The number of generations passed through in a given time depends upon temperature and whether the first- or last-born young are selected to carry on the next generation.

sexuparae and males. The former occur when conditions favour parthenogenetic reproduction, when all the alatae may be virginoparae. During October and November, under natural conditions, the offspring of the apterae may develop entirely into alate sexuparae and males thus bringing parthenogenetic reproduction to an end. Under experimental conditions, the alatae which develop during the autumn and winter period may consist of a mixed brood of both sexuparae and virginoparae, or only sexuparae and males or only virginoparae depending on environmental factors, particularly temperature. It was found, for instance, that when a temperature about a mean of 70° F. was maintained, apterae predominated and the few alatae which developed were virginoparae. No case was recorded in which apterae produced sexual females as was observed by Shull(20) with *Mac. solanifolii*. The proportion of apterae which develop as offspring of apterae is affected by overcrowding, temperature and nutrition (physiological condition of food-plant). As referred to earlier, length of day (light factor) is also important in that it influences the occurrence of the sexual phase.

(2) *Offspring of the alienicolae alatae.*

The alate virginoparae are usually produced by apterae as explained in the previous sections. Their offspring usually develop into apterae, but alatae may also develop if the conditions are unfavourable, as for instance, when alatae are compelled to reproduce on plants which are heavily infested with aphids (overcrowding), or with poor nutrition. The offspring of alate virginoparae are, however, not so variable in this respect as is the case with the offspring of apterae. The alate sexuparae always appeared in the colonies about the end of September (except in line H) and afterwards in many generations throughout the winter period. They produced only sexual females, and no case was recorded in which alate sexuparae produced males as recorded by Shull(20) with *Mac. solanifolii*. No case was observed in which alatae produced both sexual females and virginoparae.

(3) *The effect of overcrowding on the occurrence of alatae and apterae.*

It has been frequently observed throughout these experiments, especially during the summer period, that, when a colony is started with one or two apterae, only an occasional alate form develops during the first 14 days or so, but alatae gradually become dominant as the colony increases, so that by the time the plant is heavily infested, large numbers of alatae are present.

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In order to test whether overcrowding was a factor affecting this increase in the number of alatae, 15 bean plants, one in each pot (seed planted on the same day), were divided into three series of five plants each. The plants were infected with adult apterae from line I on 3. vi. 27, all being of the same generation. In series A one individual was transferred to each plant, in series B, five individuals and in series C, ten individuals. The plants were kept covered with muslin bags and reproduction allowed to go on until 20. vi. 27, when the aphids were killed off and the number of apterae and alatae on each plant counted. The results obtained are shown in Table III.

Table III.

*Showing the effect of overcrowding on the occurrence of alatae.*

Series	Apterae	Alatae		Alate %
		Adult	Nymphs	
A	61	2	0	1
	59	0	0	
	52	0	0	
	45	1	0	
	31	0	0	
B	244	39	38	34
	208	41	45	
	179	90	46	
	177	33	24	
	165	80	71	
C	449	108	121	39
	363	133	89	
	358	48	46	
	284	216	116	
	228	101	107	

The column, *apterae*, includes adults and immature individuals obviously going to develop into apterae. The column, *alatae nymphs*, includes individuals which possessed wing pads. Younger individuals were not counted, as alatae and apterae cannot be readily distinguished in the earlier instars. Many of these younger individuals would of course develop into alatae, as is shown by the results of two other series of five plants each, which were set up at the same time. Each plant was infected with three apterae and reproduction was allowed to go on for 5 days longer than in the case of Table III. The counts of the colonies showed the percentage of alatae in the two series to be 77 and 81 respectively. The influence of overcrowding is shown by the number of *adult* alatae present. In series C (Table III) an overcrowded condition occurred

earlier in the colonies, resulting in alatae developing early, many being adult when the colonies were killed off. In the two series in which the colonies were started with three apterae, the average number of adult alatae in each colony was only fourteen, but the number of alatae nymphs, compared with series A-C, was considerably increased as the longer reproduction period allowed more of the later-born individuals, born under crowded conditions in the colony, to attain the 3rd and 4th instar stage in which the wing pads are visible. During the summer period, if overcrowding in the colonies is prevented, the offspring of the alienicolae apterae develop into apterae, but as soon as overcrowding occurs, alatae tend to predominate. In the parthenogenetic line C, a succession of colonies were maintained on beans from the beginning of May until September, overcrowding being prevented by removing the adults from time to time. The results obtained are given in Table IV.

Table IV.

*Showing offspring of apterae in successive colonies in the parthenogenetic line C when overcrowding was prevented.*

E = *Euonymus*; M = Migrants; S = Sexuparae; V = Virginoparae.

Colony (beans)	Aphids transferred (1922)	Alatae and apterae in colonies after 10 days		
		Days	Apterae	Alatae
1 (E)	17.4	19	25	4 (M)
2 (M)	5.5	17	25	0
3	19.5	9	51	0
4	25.5	11	50	0
5	6.6	12	3	0
6	18.6	16	Few	0
7	5.7	14	Many	0
8	19.7	12	Many	0
9	5.8	25	Few	Few (V)
10	21.8	21	Few	Few (S)
11	11.9	39	2	6 (S) + 6 ♂

Colony No. 1 was started with one fundatrix on *Euonymus* and No. 2 with two alate migrants from No. 1. The remaining colonies were started with one or two apterae from the previous colony. Apterae predominated until about the middle of August, from which time onwards the colonies developed more slowly and a comparatively small number of aphids was produced. This was due to the seasonal conditions, chiefly falling temperature and poorer growth of the bean plants, together with the advent of the sexual phase. Under these conditions, alatae tended to predominate

in the colonies and those which developed in September were sexuparae. It should be noted that, although alatae were not present in colonies 2 to 8 at the end of the period of days shown in column three, nymphs began to appear a few days later and, as overcrowding increased, the number of alatae increased.

It is clear that overcrowding is an important factor affecting the occurrence of alatae in the summer period when factors of light, temperature and nutrition (food plants) are favourable. The influence of overcrowding may to some extent be interpreted as a nutrition factor in that, the young growth of the plant being crowded, the aphids are forced to feed on the older tissues, and the sap of the young growth affords the best nutrition. Further, as the infestation increases, the tissues of the plant are so affected that they do not function in a normal manner and the quality and quantity of sap available is affected. Overcrowding is, however, relative and the phenomenon is not only a matter of nutrition, as overcrowding may occur in a comparatively small colony on a local area of a plant, resulting in an increase of alatae.

(4) *The effect of nutrition on occurrence of alatae and apterae.*

The writer has shown<sup>(4)</sup> that the reproduction rate of *A. rumicis* varies on different food-plants and is also affected by the physiological condition of the plant<sup>(8)</sup>. That the young growth of the bean plant affords the best nutrition for the insects is shown by the following experiment.

Two series, A and B, consisting of five bean plants each (seeds planted same day), were set up in pots. In series A the plants had normal growth, and in series B the young tops were cut off a few days before infection. On 31. v. 27 each plant in series A was infected with one adult apterous viviparous female from line I (offspring of alatae and reared to maturity on a normal bean plant). Similarly, each plant in series B was infected with one adult apterous viviparous female (offspring of alatae of same generation and reared to maturity on a bean plant having the top cut off). After 14 days' reproduction the ten plants were killed off and the aphids produced on each plant were counted. The results are shown in Table V.

It is evident that by removing the young top of the bean plant, its nutrition value for the aphid is affected. There is, however, no indication that the proportion of apterae and alatae has been influenced. It would be necessary to rear the aphids under these conditions through further generations to find out the cumulative effect, if any, of the two sets of

Table V.

*Showing the comparative reproduction rate on beans with normal growth (A) and with tops cut off (B).*

Series	Apterae	Alatae	Total aphids present	Mean
A	44	0	390	335.2
	34	0	276	
	33	0	384	
	32	0	339	
	29	5 (N)	287	
B	24	1 (N)	163	104.8
	24	0	165	
	23	0	84	
	14	0	72	
	10	0	40	

N = Nymphs.

conditions. Data available from experiments made to test the influence of different food plants and of the physiological condition of the bean plant on the reproduction rate of the bean aphid indicate that, on those plants which favour a high reproduction rate, the proportion of alatae which develop is smaller than on those plants on which a low reproduction rate occurs. This was observed, for instance, with poppies, peas, turnips and mangolds compared with broad beans, and its occurrence on *Euonymus* has been already referred to. Similar observations were made with different varieties of field beans. The available data does not allow of definite conclusions being drawn, but the factor of overcrowding does not appear to be the only one concerned, and the question as to whether the nutrition value of different food-plants affects the proportion of alatae and apterae which may develop requires further investigation. There is no doubt that the physiological condition of the food plant, in that it affects the nutrition of the insect, is a factor of importance, which must be considered when the influence of other external factors are being investigated if uniform results are to be obtained. The starvation experiments of Gregory<sup>(12)</sup> and Wadley<sup>(23)</sup> show clearly that poor nutrition of the parent female results in an increase in the proportion of alatae in the offspring.

During the winter period in the heated glasshouse, beans grow spindly and are poor plants compared with those grown in the summer months, due chiefly to the effect of temperature in relation to the winter light conditions. The aphid colonies on these winter plants progress more slowly than in summer, even when summer temperatures are maintained. The developmental period of individuals in winter, when reared under

“summer” temperatures approximated closely to that obtained with summer individuals (effect of temperature), but the aphids individually were not so prolific. Moreover, while with a mean temperature of about 60° F. in winter fewer apterae were obtained, apterae predominated under these temperatures during the summer period if overcrowding was avoided, which suggests the influence of the better nutrition value of the plants in summer. During the winter period with moderate temperatures alatae tended to predominate in the colonies, although with comparatively high temperatures (see p. 121) the influence of the nutrition factor is overcome and apterae predominate. It is interesting to note that the apterae frequently feed below the leaves on these winter plants and not on the growing apex, whereas in summer they invariably feed on the growing tip, unless overcrowded conditions force them to the older parts of the stem and beneath the older leaves. On two or three occasions during the winter period, it was observed that the aphids on bean plants which became sickly owing to root rot developing, left the plant (particularly the apex of the stem) and wandered to the muslin covers. These observations show that the aphids react to the physiological condition of the plant.

From March onwards there is a marked improvement in the growth of the bean plants compared with the earlier period of winter, and as spring advances, if a favourable temperature is maintained, the aphid colonies make better progress and a higher proportion of apterae develop.

(5) *The effect of temperature on occurrence of alatae and apterae.*

It has been shown that during the summer period when the aphids are reared on a favourable food plant (broad beans) and overcrowding avoided, the offspring of apterae tend to be predominantly apterae. Favourable nutrition, correlated with the large area of succulent growth on the plants, is the most important factor favouring the occurrence of apterae during this period, and the moderate fluctuations in the summer temperature do not markedly influence the sequence of alatae and apterae. Alatae, however, predominate irrespective of temperature when overcrowding occurs. During September, under the influence of a falling temperature, shorter hours of daylight, and lack of suitable food-plants (nutrition), the aphid colonies are much smaller and alatae tend to predominate even when overcrowding does not occur and comparatively few apterae may develop. When the aphids were transferred to a warm glasshouse in October, the proportion of apterae increased. In Fig. 3 the results are shown of counts made of apterae and alatae

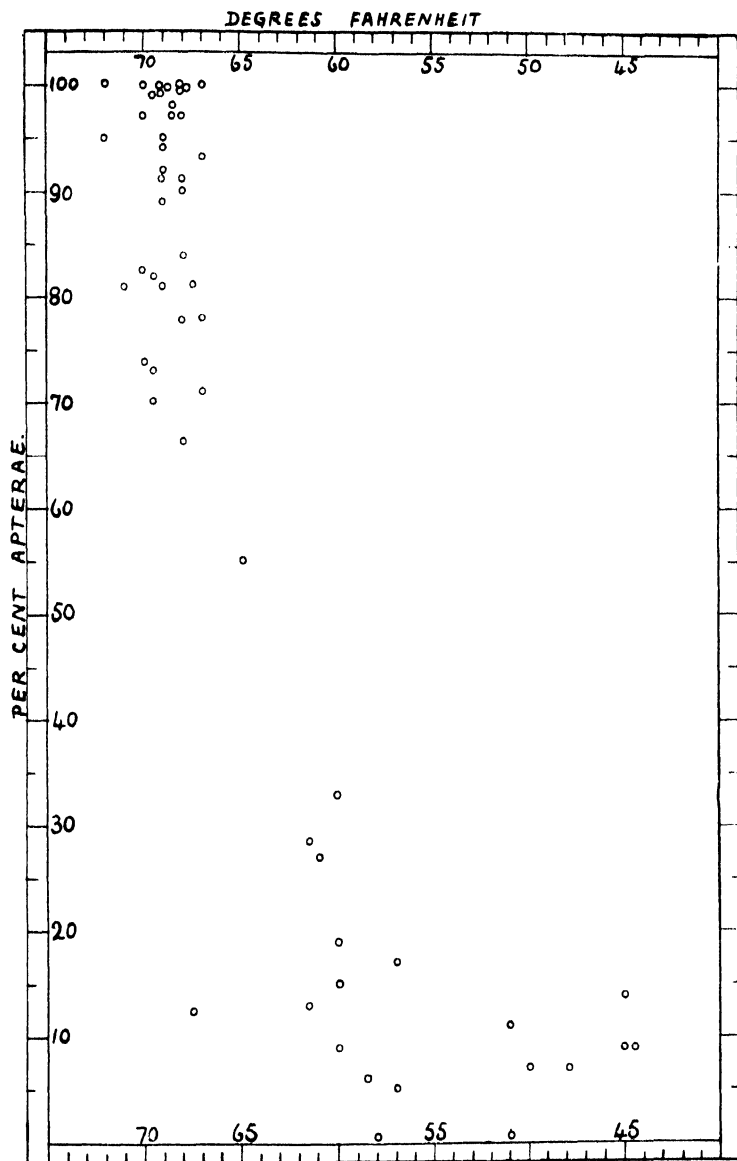


Fig. 3. Showing the influence of temperature on the occurrence of apterae and alatae during the winter period. Counts were made from 56 colonies (offspring of apterae) in lines A, B, C, G, H, Ha during the winter period and the percentage of apterae present in each colony is plotted with reference to the mean temperature of the reproduction period in each case. As far as possible precautions were taken to prevent overcrowding.

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present in 56 colonies reared under various temperatures during the winter period. The colonies were taken from five of the parthenogenetic lines in different years. They were killed off for counting after a reproduction period of 2 to 3 weeks or longer in the case of the lower temperatures. Precautions were taken to prevent overcrowding and the alatae and apterae were diagnosed as in the experiments referred to in a previous section. With a mean temperature of about 67° F. and over there was a marked increase in the proportion of apterae in the colonies, and below about 57° F. there was a relatively small proportion of apterae. It should be noted that these counts include the colonies which received artificial light, as there was no apparent difference in the proportion of apterae and alatae present compared with the control colonies, as is seen in Table VI. The temperatures maintained in both these sets of experiments were moderately high.

### B. OCCURRENCE OF THE SEXUAL FORMS.

Owing to the large number of colonies which were reared in the various parthenogenetic lines, it is not feasible to present the data in the form of tables, but in Figs. 4 and 5 the occurrence of the sexual forms has been indicated by symbols placed in positions which show the approximate dates when *adult* sexual individuals were recorded in the colonies. Each symbol represents a varying number of individuals present in a colony, so that usually the total number of symbols on any parthenogenetic line shows the number of colonies in which sexual forms were recorded.

It will be seen from Fig. 1 that, with the exception of line H in which the colonies were reared under shorter hours of daylight, *adult* sexual forms occurred with great regularity each year in October, and where the lines were continued parthenogenetically throughout the winter, they appeared from time to time from October until about the end of the following May. It is interesting to note that, although sexual forms developed during the spring and early summer months in overwintered lines, they did not develop in the colonies of lines started from the fundatrix in spring until the following October, although during the spring period both sets of colonies were reared under the same conditions. In the colonies of the overwintered lines there was, however, a progressive increase in the proportion of parthenogenetic individuals present in the colonies as spring advanced, compared with the earlier winter period: alate virginoparae began to appear during March and April, the number of alate sexuparae becoming less, so that about the end of May, the alate forms tended to be entirely virginoparae. The occurrence of the sexuales



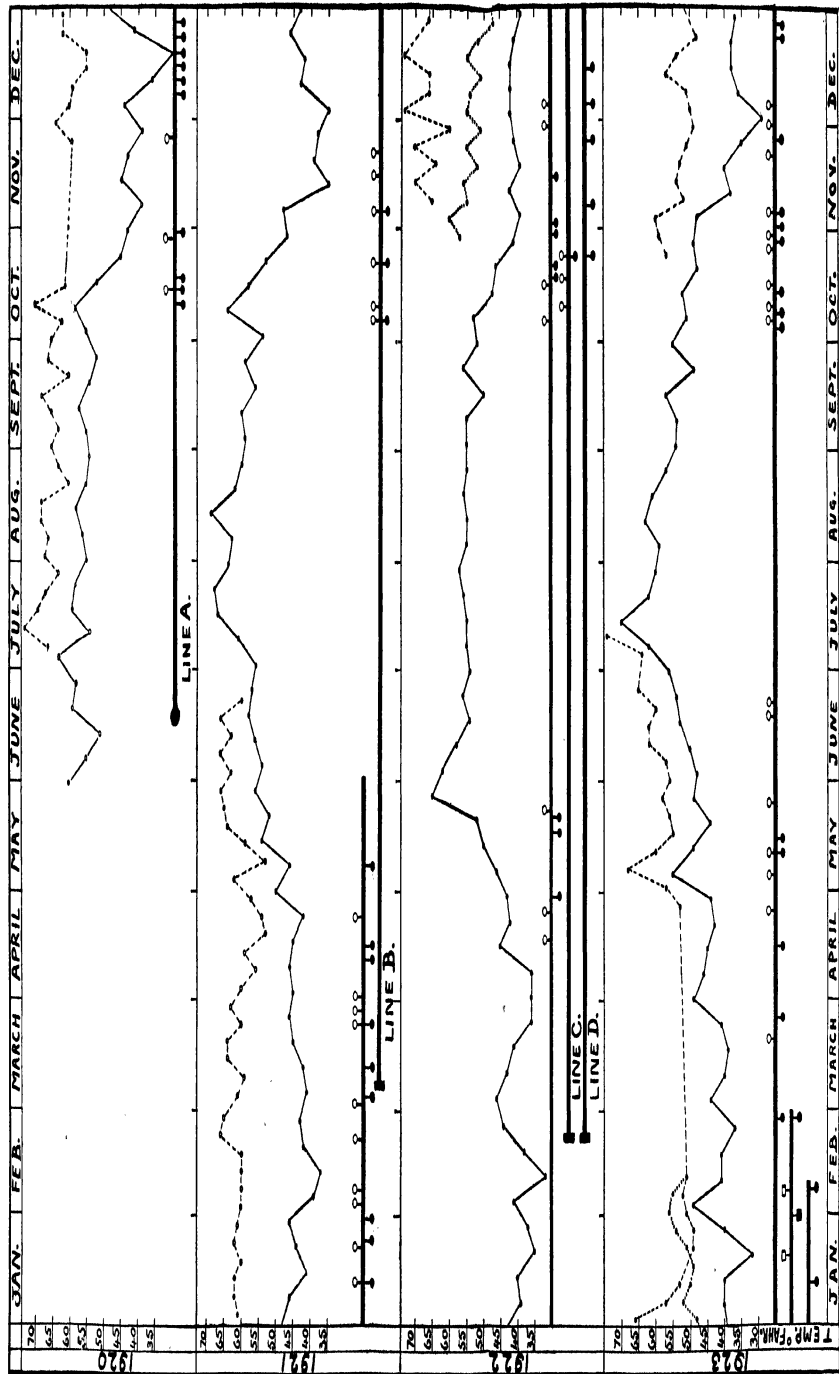


Fig. 4. The chart shows dates on which adult sexual forms of *A. fumica* were recorded in the colonies of the parthenogenetic lines A-D. The thick horizontal lines show the length of time parthenogenetic reproduction was maintained in each of these parthenogenetic lines. The symbols attached along these lines represent adult males (small open circles above the lines) and adult sexual females (small closed circles below the lines). The places where the colonies were first started are indicated by the dates these forms were recorded in the colonies. It will be noted that during June to September only parthenogenetic individuals were obtained.

The air temperature at Rothmansd (daily mean of weekly periods) is shown by the whole-line curve; the glasshouse temperature at different periods (daily mean of 6-day periods) is shown by a broken-line curve. During 12. xi. 22 to 12. i. 23 the colonies in lines B and C received artificial light (daylight during the day and artificial light at night). The colonies in lines A and D received only ordinary daylight during this same period, and lower temperatures as shown in middle (dotted line) curve (note occurrence of sexuales).



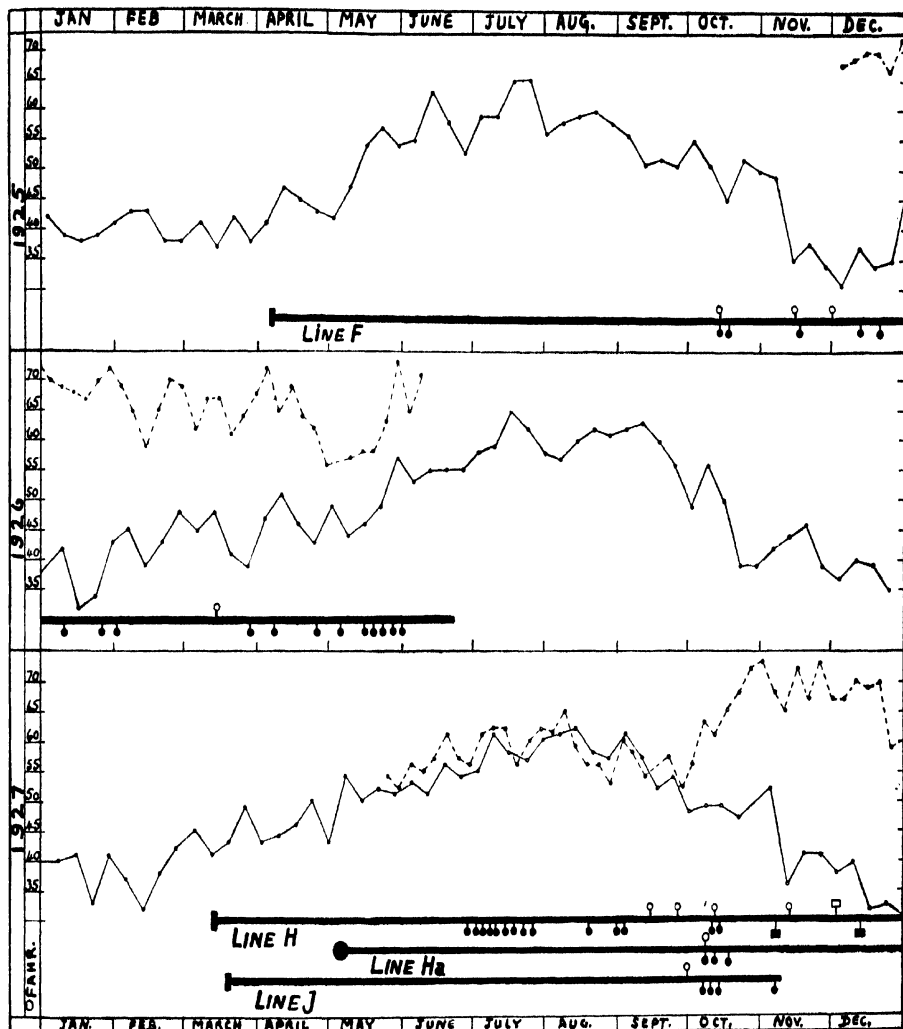


Fig. 5. The plan of this chart which shows the data obtained with the parthenogenetic lines F, H, Ha and I as described for Fig. 4. The whole-line temperature curve shows Rothamsted air temperature. The broken-line temperature curve in 1927 refers to the open-air insectary (up to 30. ix. 27) and after that date the heated glasshouse.

During the period 28. iii. 27 to 5. ix. 27 the colonies in line H received only 8 hours daylight daily (note early appearance of sexual females). During period 25. x to 23. xii. 27 the colonies in line H and Ha received artificial light in excess of normal daylight (note absence of sexuales); the square symbols indicate occurrence of sexual forms in colonies not receiving artificial light.

From particulars given in Section II of the paper, by referring to Figs. 4 and 5, the reader can see the conditions under which the aphids were reared throughout the year.

Charts IV and V should be compared with Fig. 1.

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during the spring period indicates a carrying-over effect of the previous winter conditions under which the aphids were reared.

After the sexual forms first began to appear in the various lines, apterae were transferred in successive generations to form new colonies and so continue the parthenogenetic line: the alate offspring were tested out in succeeding generations to see whether they produced sexual females (being sexuparae) or parthenogenetic females (being alate virginoparae). It is necessary in order to make sure that the sexual phase is present or absent, to rear the offspring of these alatae in as many of the successive generations as possible, in order to test whether the latter are sexuparae or virginoparae. In my experiments males were found sometimes to occur somewhat irregularly during the winter period, and one cannot rely upon their non-occurrence as indicating that the sexual phase is absent. Moreover, under the higher temperature conditions during the winter period many alatae died without reproducing, and it was necessary to isolate these forms carefully so as to ensure that they would reproduce. It is due to the precautions taken in this way and to the fact that by rearing a large number of colonies, a representative number of the insects in successive generations were available, that the presence of sexual forms has been demonstrated over such extended periods.

The details regarding the occurrence of the sexuales in the various lines are given below; the dates given are those on which the sexual forms were adult.

1. *Line A.* The sexual phase set in early in October 1920. From October until the following May, 37 colonies were reared and adult sexual forms occurred in most of them on various dates as shown in Fig. 4. After May 1921 no sexual forms occurred.

Males were first recorded on 14. x. 20 and developed in 12 later colonies, the last record being 23. iv. 21. Altogether about 50 males were recorded.

Females were first recorded on 14. x. 20. They appeared in 21 later colonies on various dates, the last date being 7. v. 21. Throughout the winter period, the alatae tested out proved to be only sexuparae, but on 23. iv. 21 both sexuparae and alate virginoparae were present and after this date only virginoparae developed.

2. *Line B.* As this line was carried on through three consecutive winter periods, it will be more convenient to deal with these three periods separately. It will be noted from Fig. 1 that there was a rhythmical

appearance of the sexual forms in October each year and a suppression of the sexual phase from about the end of May onwards, until the following October.

*First winter period 1921-1922.*

The sexual forms appeared early in October 1921 and on various dates until the following May. The temperature in the glasshouse during the greater part of this winter period was comparatively low (about a mean of 50° F.) so that the developmental period of the aphids was long and only 12 small colonies were reared. This explains the few records for sexual forms which were obtained, but the sexual phase was in evidence throughout the period.

Males were first recorded on 6. x. 21 and they occurred in eight subsequent colonies, the last record being 23. v. 22. About 24 individuals were recorded.

Females were first recorded on 6. x. 21 and they developed in five subsequent colonies, the last record being 18. v. 22.

*Second winter period 1922-1923.*

Sexual forms again appeared in October 1922, being present in six colonies during this month and the early part of November. During the period November 8th, 1922 to January 12th, 1923, high temperatures were maintained (see Fig. 4), and the successive colonies (ten in number) received artificial light as explained in Section II. Under these conditions parthenogenetic reproduction was vigorous, apterae predominated and comparatively few alatae developed. Of the latter, some were tested out on 6. xii. 22 and 20. xii. 22 and were found to be virginoparae. With the exception of one male which developed on 29. xi. 22 and one on 6. xii. 22, no sexual forms occurred in these ten colonies.

Five control colonies reared during the same period, under the same temperature, but without artificial light, behaved similarly and no sexual forms developed in them. The results obtained with these control colonies are given in Table VI, together with those of six of the colonies which received artificial light, to show the number of individuals produced under these conditions. These data have been included in Fig. 3.

Five apterae were isolated from one of these control colonies on 6. xii. 22 and placed under lower temperature (middle dotted line in Fig. 4). The majority of their offspring developed into alate sexuparae. It should be noted that even with high temperatures during the winter period—which favours parthenogenetic reproduction—the winter light

Table VI.

*Showing the proportion of apterae produced in colonies of line B during winter period 1922-1923.*

A.L. = artificial light series; N.L. = controls with no artificial light.

Colony no.		Infection with apterae	Repro- duction period days	Mean temp. of period ° Fahr.	No. of aphids		Apterae %
A.L.	N.L.				Apterae	Alatae	
51	—	2	21	67	32	0	100
59	—	2	14	68	52	8 (N)	88.6
—	48	2	14	68	20	2 (N)	91
—	54	2	14	68	10	0	100
45	—	5	14	68	40	5	89
60	—	2	14	68	44	0	100
—	49	2	14	68	35	1	97
—	55	2	21	67	25	2	93
52	—	2	21	69	86	8 (N)	91.4
61	—	2	14	67	48	0	100
—	50	2	14	67	20	8 (N)	71.4

conditions tend to exert an influence in favouring the occurrence of the sexual phase as will be discussed later.

After the middle of January 1923 the aphids in line B were reared under lower temperatures. From the middle of January until June 1923, eleven colonies were reared in which males developed on various dates, the first being recorded on 20. iii. 23, the last record being 22. vi. 23. Altogether about 26 males were recorded in this second winter period. Females also developed in five of the eleven colonies referred to above, the first record being 28. ii. 23 and the last 15. v. 23. The alatae tested out after 24. v. 23 proved to be only virginoparae and no sexual forms occurred in the colonies until the following October (third winter period).

#### *Third winter period 1923.*

Males were first recorded on 6. x. 23 and they appeared in seven further colonies on various dates, about 47 individuals being recorded. Females were first recorded on 3. x. 23 and subsequently in six further colonies. The parthenogenetic line was discontinued at the end of December 1923. It will be noted (Fig. 4) that the temperature conditions during this winter period were more favourable for the occurrence of sexual forms than was the case with the somewhat higher temperatures maintained during November and December of the previous winter.

3. *Line C.* Sexual forms appeared in this line in October 1922. During the period November 8th 1922 to January 12th 1923 five

colonies were reared under similar temperatures and artificial light as in line B. The results obtained were similar, sexual forms did not develop, but females occurred later in a continuation colony from this series on 28. ii. 23. Six control colonies were reared during this period under similar conditions but without artificial light, and no sexual forms occurred. However, they appeared earlier in the colonies descended from this control series than in the colonies descended from the series which received artificial light. One male developed on 19. i. 23 and another on 7. ii. 23 and females developed on 31. i. 23. These cases are shown in Fig. 4 by square symbols. Altogether 8 males were recorded in line C. The parthenogenetic line was discontinued at the end of February 1923.

4. *Line D.* In this line adult females were first recorded on 24. x. 22 and they developed in five subsequent colonies, the last record being 8. ii. 23. No males were recorded which is probably due to the fact that only a few colonies were reared and the number of aphids produced was small owing to the lower temperatures (see Fig. 4, middle dotted line). It is interesting to note that, under these lower temperatures, the alatae which developed were only sexuparae and sexual females were produced throughout the period.

5. *Line E.* Males and females were recorded in October and November 1924, and the parthenogenetic line was discontinued at the end of the latter month.

6. *Line F.* Sexual forms appeared about the middle of October 1925 and occurred during November. From 1. xii. 25 onwards throughout the winter period, twenty-six colonies were reared, and sexual females developed at frequent intervals as shown in Fig. 4, the last record being 30. v. 26. The alatae tested out from time to time proved to be sexuparae, but in two colonies on 8. iv. 26 and 29. iv. 26 alate virginoparae were also present and after 17. v. 26 only virginoparae developed, the sexual phase being suppressed. It was particularly noted in this line, during the period December to March, that many alatae died without reproducing, and careful attention was necessary in order to get the alatae to reproduce. In one instance, about 14. xii. 25, one plant was infected four times with a total of 32 alate forms and only 4 individuals were produced, which were sexual females. The temperature conditions evidently favoured parthenogenetic reproduction and the sexual forms obtained were few in number. Even where sexuparae developed, they were induced to reproduce only with difficulty. From December 1st onwards two isolated males were obtained, one on 2. xii. 25 and the other on 15. iii. 26.

It will be noted that the temperature during this winter period was only slightly lower than during the period November 1922 to January 1923, when, in lines B and C, the sexual phase was practically suppressed in the control colonies and completely suppressed in those colonies which received artificial light.

7. *Line G.* Sexual forms appeared in October 1926 and the parthenogenetic line was discontinued early in November.

8. *Line H.* Under the conditions of short hours of daylight in this line (see Section II) adult sexual females were first recorded on 29. vi. 27. From 18. vi. 27 to the beginning of October, eighteen colonies were reared and females occurred in thirteen of these, the last record being 15. x. 27. It was noted that from the beginning of June the offspring of the apterae tended to be alatae, which proved to be sexuparae. Males, on the other hand, were not recorded until 14. ix. 27, that is, about the normal time, others were recorded on 26. ix. 27 and 15. x. 27, in all a total of five individuals.

From October 6th onwards eight colonies were reared under higher temperatures and received artificial light as stated in Section II. Under these conditions parthenogenetic reproduction was vigorous and the aphids which developed were in a large majority apterae. The comparatively few alatae which developed were found to be virginoparae, and no sexual forms were obtained. Nine control colonies were also reared under the same temperatures, but without artificial light. Apterae predominated also in these control colonies and comparatively few alatae developed; those tested out on 15. x and 2. xii. 27 proved, however, to be sexuparae and sexual females developed on 5. xi and 13. xii. 27. One male was recorded on 2. xii. 27. These three instances are shown in Fig. 4 by means of square symbols.

These results support those obtained under similar conditions during November and December 1922 in line B. The evidence from line H shows that favourable temperatures and long hours of daylight favour parthenogenetic reproduction, whereas the reverse conditions favour the sexual phase. Even with favourable high temperatures during the winter period (short hours of daylight), although the temperature favours parthenogenetic reproduction, there is a tendency for the sexual phase to develop, presumably due to the light factor, as is seen in the behaviour of the control colonies in this line.

9. *Line Ha.* This is a control for line H, the aphids being descended from the same fundatrix. Sexual forms did not develop in the colonies

until the middle of October 1927. Females were recorded in three colonies on October 8th, 10th and 17th, and the alatae tested out proved to be only sexuparae. Three males occurred in one colony on 17. x. 27. Colonies from this line were established on *Euonymus*, ova were laid and fundatrices commenced to hatch out on 25. iii. 28.

From the middle of October to the end of December, ten colonies were reared which received artificial light as in line H. The aphids in these colonies were chiefly apterae, very few alatae developed and those tested out on 16. xi, 12. xii and 15. xii. 27 proved to be virginoparae. No sexual forms were obtained.

Eight control colonies were reared during the same period as in line H. Parthenogenetic reproduction was vigorous and no sexual forms were obtained.

10. *Line I.* In this control line sexual forms did not occur until October, females being recorded in four colonies about 10. x. 27 and 14. xi. 27. Three males developed in one colony on 30. ix. 27. The line was discontinued about the middle of November.

## VII. GENERAL CONCLUSIONS AND SUMMARY.

### 1. *Material employed in the experiments.*

Ten related lines of the black bean aphid (*Aphis fabae* Scop. = *A. rumicis* L.), in which continuous parthenogenetic reproduction was maintained for varying periods, have been reared during the past seven years (Fig. 1). The longest period was 2 years 10 months with line B, 50 generations having been passed through. The lines were all of the same strain, nine of them being descended from line B, which was started from one fertilised egg. The remaining lines (except Ha) were similarly started from fertilised eggs.

*Euonymus europaeus* was used as the primary food-plant (winter host) and broad beans as the intermediate (summer) food-plant. During the summer period (April to October) the aphids were reared in a large open glasshouse or in the open-air insectary, and during winter (November to March) in a heated glasshouse with varying temperatures (Figs. 4 and 5). The *Euonymus* plants on which fertilised eggs were laid in autumn were kept outside in the open during the winter period.

## 2. *The normal life cycle of Aphis rumicis L.*

*Aphis rumicis* is a migrating species in England, its usual winter host being *Euonymus europaeus*. Its normal life cycle resembles that of other migrating Aphidini; sexual forms occur about October and the fertilised eggs commence to hatch the following March (Fig. 2).

## 3. *Fundatrigeniae generations.*

The fundatrix is considered in this paper as the first generation. Its offspring are usually apterae but may also include alatae (migrants). The apterae of second generation give rise chiefly to alate migrants and a few apterae. The offspring of the latter forms normally consist of alate migrants so that the fundatrigeniae generations come to an end.

There is an inherent tendency for the offspring of the fundatrigeniae apterae to develop into alate migrants, evidently associated with the evolution of the migrating habit. The proportion of alatae and apterae is, however, influenced by environmental factors, particularly overcrowding and the condition of the food-plant (nutrition)<sup>1</sup>.

## 4. *Alienicolae generations.*

The alate migrants have a strong inherent tendency to migrate from the primary host plant, but if confined on *Euonymus* they reluctantly reproduce on it. Moreover, while their offspring on beans develop into apterae, when produced on *Euonymus* some of them develop into alatae owing to the influence of the nutrition factor. The migrants initiate the alienicolae generations. Under certain environmental conditions the succeeding alienicolae generations may consist only of parthenogenetic individuals, namely apterae and alate virginoparae. Under other conditions sexual forms develop. The latter consist of alate males (offspring of apterae) and apterous females (offspring of alate sexuparae). The proportion of sexual and parthenogenetic individuals which develop depends upon environmental factors: on the one hand we may get the parthenogenetic generations terminated owing to the fact that sexual individuals only are produced; on the other hand, only parthenogenetic individuals may be produced. Conditions may occur under which both types are represented.

<sup>1</sup> In non-migrating species, *migrantes* in the true sense do not occur and the *alatae* are to be considered as dispersal forms. Reinhard (19) considers that with *Aphis gossypii* the normal tendency is for the parthenogenetic individuals to be apterous. Baker and Turner (2) showed that with *Aphis pomi* the complete bi-sexual cycle could be completed without the occurrence of alate individuals.

## A. THE OCCURRENCE OF ALATAE AND APTERAE.

Three types of alate parthenogenetic females occur during the complete life cycle, namely (1) migrantes, (2) dispersal forms, (3) sexuparae. These three forms resemble one another morphologically, but they differ in that each type plays a different rôle in relation to the migrating habits of the insect. It is necessary therefore to consider them separately when dealing with the influence of various factors on the occurrence of apterae and alatae.

The migrantes have been dealt with in the previous section as they belong to the fundatrigeniae generations. The dispersal forms and the sexuparae occur in the alienicolae generations.

The alate dispersal forms (alate virginoparae as distinct from the alate sexuparae) are chiefly offspring of apterae. They ensure a wide distribution of the species, but are not essential for the completion of the bi-sexual cycle. Their occurrence depends upon environmental factors, particularly (1) overcrowding, (2) temperature, (3) nutrition. Correlated with their function as "colonisers," their offspring normally develop into apterae which latter are more prolific than the alate forms. Under favourable conditions the offspring of apterae develop into apterae, but with adverse conditions of overcrowding, low temperatures and poor nutrition, alatae may also develop. The proportion of apterae and alatae depends upon the influence of these three factors. Actually during the summer, temperature does not play such an important part owing to the moderate fluctuations about a favourable mean temperature.

The experimental results obtained by Wadley<sup>(23)</sup> and others with other species of aphids show that under the influence of one or more of the factors referred to above, the offspring of apterae (and to some extent alatae) may develop into apterae or alatae. The fate of the individuals in this respect may be determined in the early instars immediately after birth, or during pre-natal development in the parent female. From the embryological studies of Uichanco<sup>(21)</sup> we know that the embryos of the young offspring are well advanced, even in the early instars of the parent female. With the exception of the work of Ackerman<sup>(1)</sup> no detailed investigations have been made on the physiological aspect of the occurrence of apterae and alatae, and it is to be hoped that his interesting observations will be studied further.

The alate sexuparae mark the appearance of the sexual phase. The occurrence of the latter is associated with the seasonal factors of light (length of day) and temperature.

## B. THE OCCURRENCE OF THE SEXUALES.

The rhythmical occurrence of the sexuales in October each year in the various lines (Figs. 4 and 5), indicates that this phenomenon is due to the influence of seasonal factors. Light (length of day) and temperature have been shown to be the important factors. While with normal daylight adult sexuales first occur in October, it was found that in the case of line H, in which the colonies received only 8 hours daylight daily, sexual females first appeared in June and were obtained in subsequent generations throughout the summer.

When the various lines were continued parthenogenetically throughout the winter in a heated glasshouse using normal daylight, sexual forms appeared in the colonies until the following May, provided a moderate temperature (about a mean of 58° F.) was maintained (lines A and D). From March onwards the proportion of parthenogenetic individuals increased and with the exception of two instances in early June, adult sexuales were not obtained during June to September (excluding line H), but they reappeared again in October (line B).

With moderately high temperatures during the winter period (about a mean of 70° F.) sexual forms were obtained only rarely or not at all (lines B, H, indicated by square symbols). There was a high proportion of parthenogenetic individuals under these conditions and apterae predominated.

With slightly lower temperatures during the winter period (line F) a few sexual females were obtained in most generations, but the alate sexuparae did not readily reproduce. Males were rare.

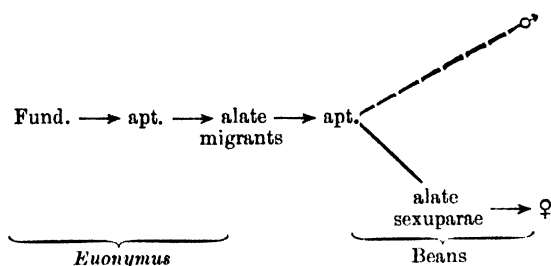
When the colonies were subjected to artificial light in excess of the normal daylight during the winter period, and a mean temperature of about 70° F. maintained, no sexual forms appeared (lines B and C, November 1922 to January 1923 and lines H and Ha, October to December 1927).

The higher temperatures favour the development of parthenogenetic forms, while the winter light conditions favour the development of the sexual forms. The normal parthenogenetic method of reproduction during the summer, and the suppression of the sexual phase is to be considered as an expression of the influence of these favourable seasonal factors<sup>1</sup>.

<sup>1</sup> The few records of the occurrence of sexuales in tropical and sub-tropical countries does not necessarily indicate a complete absence of these forms in most species. Owing to the favourable environment, parthenogenetic forms may be dominant, and the sexuales comparatively few. Yingling (*Journ. Econ. Entom.* p. 223, 1917) records the sexual females and eggs of *Anoecia corni* in South Texas during December.

From the cytological studies of Morgan, von Baehr, and others it is evident that the mechanism which determines the change from the parthenogenetic to the sexual individual, is associated with the chromosomes. The fate of the offspring of the sexuparae is therefore determined during their pre-natal development. Thus, for instance, although adult sexual forms occurred up to about the end of May, they were probably determined by the conditions obtaining about the end of April. When adult alate sexuparae were isolated in July from colonies in line H and placed under normal daylight conditions, their offspring developed into sexual females. Similarly, during the winter period, when alate sexuparae, reared under temperatures about 58° F., were transferred to higher temperatures, their offspring developed into sexual females.

The fundatrix is also predetermined in the fertilised egg, and Baker and Turner(2) have shown with *Aphis pomi* that the embryo is well advanced by the time the fertilised egg assumes the winter resting condition. With migrating species of aphids like *A. rumicis* it is possible that not only is the fundatrix predetermined but also the occurrence of the alate migrants is inherently established. This would explain why sexual forms did not appear in line H until June. Actually in this instance alate sexuparae developed in the second alienicolae generation as shown below.



Normally, in nature, many alienicolae generations are passed through before the sexuales appear in autumn, and the results referred to above show clearly that the sexual phase develops irrespective of the number of generations passed through. The seasonal factors concerned in affecting the occurrence of the sexual and parthenogenetic phases in the life cycle of *A. rumicis* are shown graphically in Fig. 6, and the dates of the

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occurrence of sexual forms in the various lines should be referred to this diagram.

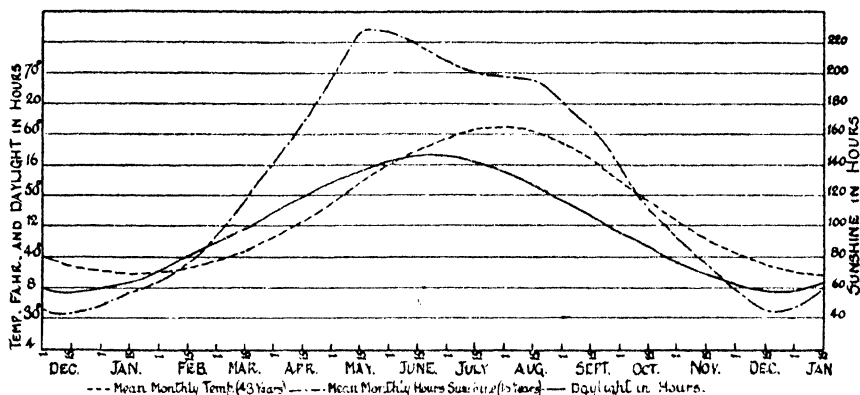


Fig. 6. Diagram illustrating the relation of seasonal factors of temperature and sunshine (Rothamsted Records) and daylight throughout the year. The dates of the occurrence of sexual forms in the various lines of *A. rumicis* should be referred to this diagram.

### SUMMARY.

1. Ten related lines of *Aphis rumicis* L. have been reared in which parthenogenetic individuals were obtained in successive generations extending over varying periods (Fig. 1): the longest period was 2 years 10 months (line B), 50 generations having been passed through.

2. The aphids were reared in an open-air insectary or a large, open glasshouse during April to October (summer period) and in a heated glasshouse with varying temperatures (Figs. 4 and 5) during November to March (winter period): *Euonymus europaeus* was used as the primary host and *Vicia faba* as the intermediate food plant.

3. The life cycle of this species resembles that of the normal type of migrating Aphidini: in S.E. England the fundatrix usually hatches in March and the adult sexuales appear in October.

4. Three types of alate parthenogenetic females occur during the complete cycle, namely migrantes, dispersal forms and sexuparae: these forms resemble each other morphologically but differ in their relation to the migrating habits.

(a) The migrantes may develop as offspring of the fundatrix, but more usually they are offspring of the fundatrigeniae apterae: their occurrence is due to some extent to an inherent established tendency, associated with the migrating habit, but the numbers which may occur in the various fundatrigeniae generations is influenced by overcrowding

and the condition of the food plant (nutrition): they normally produce apterae (alienicolae).

(b) The alate dispersal forms are usually offspring of alienicolae apterae, but may also be produced by the alatae: their occurrence in the various alienicolae generations is affected by overcrowding, nutrition and temperature: they normally produce apterae, but under adverse conditions may also produce alatae.

(c) The alate sexuparae are offspring of alienicolae apterae and their occurrence marks the appearance of the sexual phase; they produce sexual females.

5. With suitable environmental conditions the parthenogenetic alienicolae generations may be maintained experimentally for long periods, and the sexual phase may be induced or suppressed depending on the factors of light (length of day) and temperature. The effect of nutrition (if any) due to the influence of these factors on the plant has not been determined.

6. With the normal seasonal hours of daylight, adult sexual forms appeared regularly in the various lines in October each year. Moreover, they were obtained throughout the winter period until the following May when moderate mean temperatures (about 58° F.) were maintained (lines A and D); with higher mean temperatures (about 70° F.) during this period, they only occurred occasionally (lines B and C, November 1922 to January 1923, and lines H and Ha, November to December 1927); with slightly lower mean temperatures (line F) a few sexual females were obtained in every generation, but males were rare.

7. When the colonies received only 8 hours daylight daily from the fundatrix stage onwards (line H), sexual females developed in June and subsequent months; males did not appear until October.

8. When colonies received artificial light from electric lamps, in addition to the normal hours of daylight, during the winter period (lines B and C, November 1922 to January 1923, and lines H and Ha, November to December 1927) no sexual forms were obtained; the mean temperature was about 70° F. and, as stated in paragraph 6, the sexual forms only occurred in isolated instances in the control plants (received no artificial light) owing to the influence of the high temperature.

## REFERENCES.

This list contains only the publications referred to in the present paper. Further references to papers dealing with the influence of external factors on the occurrence of the parthenogenetic and sexual forms will be found in several of the papers given below.

- (1) ACKERMAN, LLOYD (1926). *Journ. Exper. Zoology*, XLIV, 1-60.
- (2) BAKER, A. C. and TURNER, W. F. (1916). *Journ. Agric. Res.* v, 955-93.
- (3) BRITTAIN, W. H. (1922). *Proc. Acadian Entom. Soc.* 1921, 7-29.
- (4) DAVIDSON, J. (1921). *Ann. App. Biol.* VIII, 51-65.
- (5) — (1921). *Sci. Proc. Royal Dublin Soc.* XVI, 304-22.
- (6) — (1921). *Bull. Entom. Res.* XII, 81-9.
- (7) — (1924). *Science*, LIX, No. 1529, p. 634.
- (8) — (1925). *Ann. App. Biol.* XII, 472-507.
- (9) — (1926). *Verh. 3ten Intern. Ent. Kongress, Zurich 1925*, II, 452-7.
- (10) — (1927). *Journ. Linnean Soc. London, Zoology*, XXXVI, 467-77.
- (11) EWING, H. E. (1925). *Amer. Nat.* LIX, No. 663, pp. 311-26.
- (12) GREGORY, L. H. (1917). *Biol. Bull.* XXXIII, 296-303.
- (13) HORSEFALL, J. L. (1925). *University of Iowa Studies in Nat. Hist.* XI, No. 2 (New series, No. 87), 1-57.
- (14) MARCOVITCH, S. (1923). *Science*, LVIII, 537-8.
- (15) — (1924). *Journ. Agric. Res.* XXVI, 513-22.
- (16) — (1925). *Ibid.* XXX, 441-9.
- (17) MASON, A. C. (1922-3). *Florida Entomologist*, v, 53, 52; VI, 25-32; VII, 1-7.
- (18) PADDOCK, F. B. (1919). *Texas Agric. Expt. Sta. Bull.* 257, 1-54.
- (19) REINHARD, H. J. (1927). *Ibid.* 353, 5-19.
- (20) SHULL, A. F. (1918). *Amer. Nat.* LII, 507-20.
- (21) UIOHANCO, L. B. (1921). *Psyche*, XXVIII, 95-109.
- (22) — (1924). *Philippine Journ. of Science*, XXIV, 143-247.
- (23) WADLEY, F. M. (1923). *Ann. Entom. Soc. America*, XVI, 279-303.

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# THE INTERNAL CONDITION OF THE HOST PLANT IN RELATION TO INSECT ATTACK, WITH SPECIAL REFERENCE TO THE INFLUENCE OF PYRIDINE

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## PART I. THE NATURE OF THE PROBLEMS INVOLVED<sup>1</sup>.

By J. DAVIDSON.

THE study of the internal condition of the food plant in relation to insect attacks has received very little attention on the experimental side, and a wide field is open for research on these lines. After a lengthy investigation of the factors affecting the infestation of the tea plant by *Helopeltis theivora*, Andrews<sup>(1)</sup> concluded that the vitality of this insect is directly controlled by the suitability or otherwise of the food supply. Withycombe<sup>(11)</sup> in Trinidad has shown that sugar cane growing on heavy clay soils, having badly aerated roots and a marked susceptibility to water shortage, more readily succumbs to the attack of the frog-hopper *Tomaspis saccharina*. Davidson<sup>(3)</sup> showed experimentally that there was a marked variation in the intensity of reproduction of *Aphis rumicis* on *Vicia faba* depending upon the nutrients supplied to the plants. Lees<sup>(5)</sup> refers to several instances which indicate the varying susceptibility of plants to insect attack due to the influence of soil conditions and growth of the plant.

The association of particular species of insects with particular food plants has doubtless resulted in an adaptation on the part of the insect, especially with regard to the physiology of digestion, in a manner best suited to its requirements. Many insects fail to live, or at any rate develop abnormally and only multiply at a markedly reduced rate, on

<sup>1</sup> The observations and experimental work on this subject were made at the Rothamsted Experimental Station while the writer was a member of the staff of the Entomological department.

other than their normal food plants as the writer(2) has shown to be the case with the polyphagous aphid *A. rumicis*. In some cases the specificity of the food plant is so definite that certain insects inhabiting one variety of a plant will fail to live on a closely related variety. One of the best known instances of this is the resistance of certain varieties of vines to Phylloxera. Trägårdh, as referred to by Müller(6), records that apple trees may differ from 2.5 to 80 per cent. in the degree to which they are attacked by *Argyresthia conjugella*.

The resistance or immunity of a plant to insect attack may be due to particular features of its morphology or anatomy. In most instances, however, it appears to be due to features closely associated with the physiology of the plant, probably the presence or absence of particular substances in the tissues of the plant which render it unsuited to the insect. For instance, the larvae of *Pieris brassicae* and *P. rapae* appear to feed on those plants whose leaves contain a particular glucoside, one of the mustard oils, and it has been shown by Verschaffelt that when this substance was smeared on leaves which the caterpillars normally avoid, they were attracted to feed on them. The chemotropic influence and other factors which enable adult insects to select their appropriate food plants is little understood, and would be a profitable line of enquiry.

It is interesting to note that certain alkaloids, which when extracted from plants are toxic to insects, are not so in their natural state in the living plant. For instance, the tobacco plant is visited by cut worms, caterpillars and plant-sucking insects, yet nicotine which is extracted from it is a potent insecticide.

It may well be that the presence of certain chemical substances in the juices of plants render them unsuited to the requirements of certain insects, but no clear experimental proof of this with reference to insect-attack, so far as I know, has been brought forward. By inoculating the juices from one plant containing particular chemical compounds into another plant known to be lacking in those substances, one might expect the substances would be taken up into the juices of the latter plant, at any rate in so far as its physiological reaction would allow of this taking place. With this point of view, Müller(6) injected extract of tomato rhubarb and tobacco sap into the stem of an apple tree infested with *Eriosoma lanigerum* in May and June, but there was no evidence that the aphid was affected by this treatment.

Grafting would appear to be a more reliable method of obtaining a transference of particular substances from one plant to another. Roach(8), who cites several authors, states, "It is known that grafting together

portions of two different plants is sometimes followed by changes in their chemical contents owing to permeation of each by substances formed in the other." He refers to the work of Brown (1926), who showed that the mineral composition of the fruit of the apple varies according to the stock on which the variety is grafted, and Barker and Grove (1914), who found that the acidity and probably the tannin content are similarly affected.

The so-called "*incompatibility*" between scion and stock, when for instance certain varieties of pears are grafted on certain quince stocks, which is being investigated at East Malling Research Station is of interest in this respect. The results of research on the physiological and biochemical aspect of grafting should be of great interest to entomologists in relation to the association of certain insects with particular varieties of fruit trees.

In a recent paper Dontaho Kostoff<sup>(4)</sup> states: "In grafting certain species and genera of the Solanaceae, mutual induction of antibodies in scion and in stock was found." "Physiological interaction between scion and stocks accompanied by different morphological phenomena in the scions and stocks were observed, which are explicable by postulating antibody production."

From the entomological standpoint, attempts to confer on plants temporary immunity from insect attack have been based on the view that the plant may be induced to take up, and absorb into its tissues, traces of certain chemical substances which, although innocuous to the plant concerned, would by their presence render it unsuited or even toxic to the insects infesting it.

It is clear that the results of any attempt to influence the growing plant as indicated above must be closely bound up with the physiological processes of the plant, and it is not surprising to find that conflicting results have been obtained by different investigators. In a recent monograph entitled *Die innere Therapie der Pflanzen*, A. Müller<sup>(5)</sup> has reviewed the subject very thoroughly and shown how complicated are the many factors concerned. This monograph is a noteworthy contribution to a wide subject which has been comparatively little explored.

The methods which have been employed by various workers in introducing chemical substances into plants may be briefly grouped under two headings:

(a) By injection of substances in solution into the stem or roots of plants (usually trees) or by introducing solid substances (*e.g.* potassium cyanide) into holes in the stem.

(b) By introducing the substances either as solids or as solutions into the medium in which the plant is growing, the substances being taken up in the usual way through the roots of the plant.

The former method has been used by several workers with the object of ridding trees of borers, aphids, and scale insects. In the case of solutions, holes are bored into the stem in which a tube is inserted, the solutions being led into the tree from a suitable reservoir. The staining of the wood of living trees on this principle has long been practised and various types of apparatus have been devised for introducing solutions into trees. Under the influence of the transpiration current, a fairly uniform distribution of the solutions throughout the tissues is obtained. The wood when seasoned is used for making furniture. Aniline dyes and those of the phenol group have been chiefly used at concentrations of 0.5 to 1 per cent., about 600 litres of solution being required per cubic metre of the tree. Dementiev (*Abstr. R.A. Entom. A.* III, 1915, 394) introduced a solution of barium chloride 1 : 350 into apple trees infested with woolly aphid in July 1903. He records that after nine days the aphids had been practically all killed. Müller(c) tried similar methods against *E. lanigerum* using solutions of chloral hydrate, nicotine muriate, and pyridine. The latter gave good results. Marked branches of apple trees treated with pyridine on 8. vi. 22 were found to be freed from the aphids on 7. vii. 22.

Employing similar methods, other chemical substances in solution have been tried by different workers, but in many cases the results have either been negative, or the resulting damage to the plants, due to the treatment, has ruled out the method as of no practical value. It is clear, however, that meteorological conditions, time of the year (season), age and type of tree, and concentrations of the substances used, are important factors affecting the results obtained.

The introduction of solid substances into the stem of the plant has the advantage of being a convenient and simple method. Sanford (*Science*, Oct. 9th, 1914, No. 1032, 519) treated Spanish broom infested with *Icerya purchasi* by boring a hole three inches deep, diameter three-eighths inch, into the stem and filling it with crystals of potassium cyanide. In a few days the scale was killed and the tree flourished. Shattuck (*Science*, Feb. 26th, 1915, No. 1052, 324) states that large groves of elms and black locust trees were successfully treated by this method against attacks of borer and girdling insects. Moore and Ruggles (*Science*, July 2nd, 1915, No. 1070, 33) however obtained negative results with this method against wood borers in oak trees. Müller (p. 49)

refers to the work of Mokrzeki, who successfully treated apple and pear trees infested with *Lepidosaphes ulmi* and *Diaspis fallax* with iron sulphate solution.

The method whereby the chemical substances concerned are added to the soil in which the plants are grown is a more normal and convenient one. There are some interesting examples in the literature showing the curative effect on plants of adding particular substances to the soil. The best known case is probably that of chlorosis due to deficiency in the soil of iron or manganese. Samuel and Piper<sup>(9)</sup> have shown conclusively that the "Grey Speck" disease of oats in certain areas of South Australia is due to a manganese deficiency in the soil, and by the incorporation of manganese sulphate in the soil the disease can be completely cured. Andrews<sup>(1)</sup>, working with *Helopeltis theivora* infesting the tea plant in North-east India, found "That when a constant supply of soluble potash is applied to the roots of the (tea) bush, this substance is taken up and the bushes which are entirely shut up by the pest can be caused to throw it off entirely, and remain immune from attacks for the rest of the season." Brenchley and Warrington at Rothamsted and others have shown the stimulating effect on plant growth of minute doses of certain chemical substances, *e.g.* Boron, which in slightly stronger doses are toxic to the plant.

Müller<sup>(6)</sup>, working with "Blumenbohnen" in water culture, tested the effect on the plants of a range of substances in various concentrations. He found for instance that with pyridine 1 : 500 and barium chloride 1 : 5000, after 5 and 17 days respectively, no ill-effects were noted on the plants<sup>1</sup>.

In 1924 the writer made some preliminary experiments in which solutions of various substances were inoculated by means of a hypodermic syringe into the stems of growing plants of *Vicia faba* infested with aphids. The following substances were used, strychnine, atropurine, digitalin, arsenic, potassium cyanide, nicotine, and pyridine. In several cases the substances were evidently carried through the plant in an irregular manner to the growing tip, and the aphids were killed. In some cases the plant itself was also killed, pathological symptoms first showing in certain areas of the growing tip, and as in the case of arsenic gradually spreading through the whole plant. Experiments were continued the

<sup>1</sup> With  $\text{MgSO}_4$  1 : 500 and chloral hydrate 1 : 1000, it was observed that the plants made better growth and bigger root development. The writer<sup>(3)</sup> working with *Vicia faba* in water culture in 1923 also obtained marked increase in root development and growth of the plants in solutions containing increased  $\text{MgSO}_4$  as compared with the controls growing in normal culture solution.

following year by placing cut stems of *Vicia faba* plants infested with *Aphis rumicis* into solutions of various substances. The examples given in the following table of some results obtained at Rothamsted in July 1926 by Mr H. T. Pagden indicate the type of results obtained.

Solutions used	Plant no.	Date and time observations made					
		28. vii. 10 a.m.	29. vii.		30. vii.		
			10 a.m.	6 p.m.	10 a.m.	6 p.m.	
Tap water controls	1	Bean plants placed in solutions 12-14 in. in height	Plants and aphids	Normal	Normal, aphids	Normal, aphids	
	2		normal		quiet	quiet	
	3						
KCN 0.25 %	4		Aphids slightly	Aphids sluggish;	Aphids sluggish;	Many aphids dead; did not wander, but died with stylets in tissues of plant	
	5		wandering over	plants wilting and	plants wilting		
	6		plants; plants appear normal	bending over about middle of stem			
BaCl <sub>2</sub> 1 : 350	7		Aphids wandering, some dead; plants wilting	—	Some aphids dead, many wandering actively; plants with leaves badly blotched	—	
	8						
	9						
MgSO <sub>4</sub> M : 10	10		Plants and aphids	Plants normal; aphids slightly	Plants appear normal; aphids wan-	Plants appear normal; aphids actively wandering over plants and muslin cover	
	11		normal	wandering	dering		
	12						

*Note.* 100 c.c. of solution used in each case. Mean temperature throughout the period was 24.5° C. The amount of solution taken up by the plants varied. In the case of the control series, 50 c.c. were taken up during the first 24 hours, whereas in the case of the other solutions only 15-20 c.c. were taken up.

From preliminary experiments with pyridine, it was found that, when beans grown in sand culture and infested with *Aphis rumicis* were watered with pyridine solution 1 : 200 and 1 : 150, the pyridine was evidently taken up by the plant to the growing tip, and the aphids wandered from the plant about 12 hours later, the great majority of them being killed. During the first day or two after commencement of the experiment, the plants did not exhibit any marked effect of the pyridine. On examination of the roots after a few days it was found, however, that the fine rootlets were discoloured and dead. When the pyridine solution was watered to beans growing in soil, the effect on the aphids was not so quickly shown. In order to find out the strength of pyridine solution required to free the plants from the aphids and at the same time not to injure the plants, Mr Henson has carried out a series of experiments which are described in the second section of this paper. The results obtained indicate a useful line of enquiry in relation to the effect of pyridine and other chemical substances on the aphids and the physiological reaction of the plant.

## REFERENCES.

- (1) ANDREWS, E. A. (1923). *Factors affecting the control of the Tea Mosquito Bug*. Indian Tea Assn. London.
- (2) DAVIDSON, J. (1921). *Ann. App. Biol.* vii, 51-65.
- (3) ——— (1925). *Ibid.* xii, 472-507.
- (4) DONTAHO KOSTOFF (1929). *Genetics*, xiv, No. 1, 37-77.
- (5) LEES, A. H. (1926). *Ann. App. Biol.* xii, 506-515.
- (6) MÜLLER, A. (1926). *Die innere Therapie der Pflanzen*. Monogr. zur angew. Entom. No. 8, Paul Parey, Berlin.
- (7) ——— (1926). *Anzeiger für Schädlingskunde*. Jahrg. ii, Heft 12, 157-164.
- (8) ROACH, W. A. (1927). *Ann. App. Biol.* xiv, 181-191.
- (9) SAMUEL, G. and PIPER, C. S. (1928). *Journ. Agric. South Australia*, xxxi, 696, 789.
- (10) WILKINS, V. E. (1927). *Research and the Land*. H.M. Stationery Office, London.
- (11) WITHEYCOMBE, C. L. (1926). *Ann. App. Biol.* xiii, 64-108.

PART II. ON SOIL TREATMENT WITH PYRIDINE AND  
ITS EFFECT ON THE INFESTATION OF *VICIA FABA*  
BY *APHIS RUMICIS* L.

By H. HENSON.

Dr Davidson's preliminary experiments on this subject are referred to in Part I of this paper. They indicated that it was possible to administer a substance such as pyridine to a plant and that it would take this up *via* the roots and so render itself toxic to insect pests such as aphids, which live by sucking the plant juices. During the summer of 1928 a series of experiments were undertaken in order to further investigate this subject and to determine whether the method showed promise of being of any economic importance. Pyridine was chosen because it was known to give positive results and because its odour rendered its presence in the plants easily detectable. A wide field for investigation is offered by the possibility of using other substances. The whole work was done under the direction of Dr A. D. Imms and Dr J. Davidson, to whom my best thanks are due.

Three experiments were projected as follows:

(a) Administration of pyridine to infected broad beans under conditions which would prove whether or not a lethal effect, due to root absorption, could be obtained. It was also used as a preliminary investigation of the degree of concentration required.

(b) Administration of pyridine under conditions similar to the above, to determine whether a range of concentrations was available in which the pyridine was potent to the animal but harmless to the plant.

(c) An experiment in which beans not infected with aphids were given pyridine and then its effect determined by taking the dry weights of the plants treated, and of controls untreated. This experiment was also taken advantage of to determine whether a small amount of pyridine administered several times had a different effect from a larger amount administered once.

*Experiment 1 (a).*

Two series of beans were grown, one in soil and the other in sand. The sand cultures consisted of 48 pots divided into five series, four of ten each and one of eight. The pots were  $9 \times 11$  in. (inside measurements) and were each fitted with a cork and glass drain tube. Any water which drained through was always returned to its own particular pot. Three days after planting (May 11th) each pot was given 1 gm. potassium nitrate, 1.24 gm. sodium sulphate, 0.4 gm. calcium chloride, 0.5 gm. magnesium sulphate, 0.5 gm. potassium dihydrogen phosphate and a trace of iron chloride made up in tap water. Subsequently the plants were only watered as required. On May 30th each plant was infected with one apterous viviparous female aphid (*A. rumicis* L.), the plants covered with muslin bags, and the infestation allowed to proceed. The commencement of the experiment for each pot was taken as the day on which the first young were produced. Apart from a few of the controls each pot was given 18 days as a reproduction period taking the day on which the first young were produced as the starting point. On June 11th pyridine was administered to 30 of the pots. The five series into which the 48 pots were divided were as follows:

1. Ten control pots in which petri dishes containing pyridine (1 part pyridine in 200 parts water) were put on the surface of the sand as a check on the action of the vapour given off from the treated pots.

2. Eight control pots in which dishes were not present.

3. Ten pots to each of which 500 c.c. of a  $\frac{1}{2}$  per cent. solution of pyridine in water were added, i.e.  $2\frac{1}{2}$  c.c. pyridine per pot.

4. Ten pots to each of which 500 c.c. of a  $\frac{1}{4}$  per cent. solution of pyridine in water were added, i.e.  $1\frac{1}{4}$  c.c. per pot.

5. Ten pots to each of which 500 c.c. of a  $\frac{1}{8}$  per cent. solution of pyridine were added, i.e.  $\frac{5}{8}$  c.c. per pot.

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The controls were given 500 c.c. of water at the same time as the pyridine was administered to the other pots.

A table of the results is given below. The reproduction figures are given in the columns. Owing to pressure of time and the fact that pots were urgently required for the next experiments, some of the controls were examined before the expiry of their allotted 18 days. These are indicated in the columns.

Exp. 1 took place over the period May 31st–June 23rd. Mean temperature during this period was 60° F.

Table I.

	Controls		Pyridine administered		
	With vapour dishes	Without vapour dishes	$\frac{1}{8}$ c.c. per pot	$1\frac{1}{2}$ c.c. per pot	$2\frac{1}{2}$ c.c. per pot
1	388 (15 days)	365	0 d	0 d	0 w
2	391 (17 days)	385	9	0	0 w
3	398	396 (13 days)	15	0	0 d
4	413 (13 days)	411 (13 days)	46	0	0 d
5	421 (16 days)	—	59	2	0 w
6	510	566	66	6	0 w
7	518	—	70	12	0 d
8	820	747	75	23	0 d
9	878	782	169	32	0
10	Discarded	1043	295	58	4
Mean	526	587	80	13	—

It will be seen at once that the pyridine has a very marked effect on the infestation. In those cases in which the infestation was not entirely checked it seemed likely that it was due to reinfection after wandering of the aphids. Very numerous dead aphids were to be observed on all the plants which were given pyridine. The figures further indicate that the plant takes up the pyridine in direct proportion to the concentration in which it is administered.

*Notes on the effect on the plants.* Too much significance should not be placed on the following notes as the pyridine was in contact with the plant roots for a matter of five days or so, which would not be the case under field conditions. A small "w" in the columns in Table I indicates the plant to have been badly wilted. A small "d" indicates less severe damage. The roots were badly affected in all cases, being caused to go black and lose their turgidity. The effect was worse in the higher concentrations as would be expected. The wilting was probably correlated with cessation of absorption by the roots. The affected plants also tended to go lighter in colour. Of course the controls were not affected

in this manner. In a few plants the pyridine was washed out and the plants allowed to go on developing. One of these bore fruit and was still healthy on July 24th.

*Experiment 1 (b).*

Soil pots were treated in a similar fashion. Twenty-four pots were taken and divided into three series: (1) 8 controls; (2) 8 to which  $\frac{5}{8}$  c.c. pyridine per pot was administered in 500 c.c. water; (3) 8 to which  $2\frac{1}{2}$  c.c. pyridine per pot were administered in 500 c.c. of water.

Beans planted May 9th. Plants infected May 30th. Allowed 18 days as time of reproduction.

Results tabulated below (Table II).

Table II.

*Soil series.*

	Pyridine administered	
	$\frac{5}{8}$ c.c. per pot	$2\frac{1}{2}$ c.c. per pot
Controls		
1214	1202	50
1211	915	24
906	866	12 d
882	733	11
390	618	10
214	209	3
1281	128	2
Discarded	Discarded	0
Mean	871	14

Mean temperature 60° F.

It will be seen that only one plant was badly affected. In fact all the others were apparently normal. The roots also were not nearly so badly affected as in the sand pots.

Exp. 1 proves that the pyridine is absorbed by the plants and, provided a suitable concentration is used, is lethal to the aphids. The concentrations to be used in Exp. 2 lie between  $2\frac{1}{2}$  c.c. and  $\frac{5}{8}$  c.c. per pot in the soil series, and between  $1\frac{1}{4}$  c.c. and a lower limit not determined by Exp. 1 in the sand pots.

*Experiment 2 (a).*

In Exp. 2 (a), thirty sand pots were taken as before. The beans were planted in seed boxes on May 31st, transplanted to the pots on June 19th and culture media given to each pot as in Exp. 1, *i.e.* 1 gm. potassium nitrate, 1.24 gm. sodium sulphate, 0.4 gm. calcium chloride, 0.5 gm.

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magnesium sulphate, 0.5 gm. potassium dihydrogen phosphate made up in tap water. On June 25th each plant was infected with a young apterous form and left to become infested. The date on which each mother first produced young was noted and the experiment was continued for 16 days (each pot) after this date. Pyridine was administered to each pot (in 500 c.c. of water) on the twelfth day (after infection) and left in contact for four days. The pots were then washed through with tap water.

Six series of five plants each were taken:

- (1) Controls to which no pyridine was given.
- (2) Five pots to which 0.1 c.c. pyridine was given.
- (3) Five pots to which 0.2 c.c. pyridine was given.
- (4) Five pots to which 0.5 c.c. pyridine was given.
- (5) Five pots to which 0.6 c.c. pyridine was given.
- (6) Five pots to which 1.25 c.c. pyridine were given.

During the period of the experiment the temperature was abnormally high and a high reproduction rate prevailed among the aphids. Under these conditions any slight variation in the action of the pyridine produced a much more noticeable effect on the counts due to the very rapid recovery after the check on the infestation. This is suggested as the explanation of the rather aberrant figures of 617 and 580 in two of the 1.25 c.c. pots. Further, it is probable that under the hot dry conditions the plants absorbed and quickly eliminated the pyridine. No data have been obtained with regard to variation in rate of absorption by the plants or of the conditions of their responses to the presence of the poison.

A table with the infestation figures is given as before.

Reproduction period 16 days. Mean temperature July 2nd-21st 70° F.

Controls	·1 c.c. per pot	·2 c.c. per pot	·5 c.c. per pot	·6 c.c. per pot	1.25 c.c. per pot
2869	1584	681	667	181	617
2655	1842	1311	290	50	580
2297	1691	1463	415	223	56
2562	1657	Plant no good	554	242	Plant died
2908	2757	1739	206	56	3
Mean:					
2658	1906	1298	426	150	314

The actual concentration per pot cannot be ascertained owing to the amount of water in each pot being unknown. The lowest concentration used was 0.1 c.c. per pot and was actually something less than 1 in 5000.

It will be observed that the same effect was obtained as in Exp. 1. The effect on the aphids is proportional to the concentration of the pyridine. This is the case even down to the very lowest concentrations used. No clear cut range of concentrations has been obtained in which the pyridine was lethal to the aphids but harmless to the plants.

*The effects on the plants.*

In no case was a detrimental effect on the plants noticed. As the highest concentration used was 1.25 c.c. per pot this agrees almost exactly with what was found in Exp. 1, where only one plant was damaged seriously by this concentration. From casual observance there seems to be a check on growth but no actual damage. Ten plants were washed clear of pyridine and left. These were still alive and healthy on August 18th.

From these data it would seem that two courses of action could be taken to check infestation: (a) a low concentration administered several times, or (b) a high concentration (represented by the 1.25 c.c. per pot conc.) allowed to act once.

Whichever of these alternatives is chosen will depend on the effect on the plants. Further data on this point will be obtained from the dry weight experiment.

*Experiment 2 (b).*

This was another soil pot experiment which gives results in accordance with what was discovered in Exp. 1 (b).

Reproduction period 16 days. Mean temperature 70° F.			
	Controls	.8 c.c. per pot	1.25 c.c. per pot
	1496	538	544
	1123	227	1237
	333	2107	649
	1414	528	72
Mean	1091.5	850	625

*Experiment 3.*

The object of this experiment was to determine the effect of the pyridine on the plants. Beans were planted in sand pots on June 20th. On June 21st each pot was given a culture solution containing 3 gm. potassium nitrate, 3.72 gm. sodium sulphate, 1.2 gm. calcium chloride, 1.5 gm. magnesium sulphate, 1.5 gm. potassium dihydrogen phosphate (made up in tap water). Each pot had ample supplies of water.

## 470 *Condition of Host Plant in Relation to Insect Attack*

Twenty-four pots were used and divided into three series as follows:

- (1) Eight controls to which pyridine was not added.
- (2) Eight pots to which 1.2 c.c. pyridine in 500 c.c. water were added on the fortieth day after planting (July 30th).
- (3) Eight pots to which 0.2 c.c. pyridine in 200 c.c. water was added on six occasions, July 24th, 26th, 28th, 30th, August 1st and 3rd.

Each pot had a drain tube as in previous experiments and ample supplies of water were always present; the drain water was returned to the pot at intervals.

The results are given in the table as dry weights of the plants concerned.

Dry weights. Period of experiment June 20th–August 14th. Weight in gm.

	Controls	1.2 c.c. per pot given once	.2 c.c. per pot given six times
	1.9	1.45	1.55
	3.95	2.15	1.7
	4.2	2.85	2
	4.45	3	2.85
	5.3	3.5	3.9
	6.7	3.6	4.5
	8.3	4.7	4.95
	9.3	5.55	5.05
Total	44.1	26.8	26.5
Mean	5.5	3.35	3.31

It will be seen at once that a very marked effect on the plants is evident from their dry weights. This single experiment is not by any means conclusive, since the existence and extent of the subsequent recovery remain unknown and many more experiments are really required to settle these points. In this particular case no difference is to be observed between the series in which a large dose of pyridine was administered once and the series in which an equivalent amount was administered in successive small doses.

### CONCLUSION.

It is known that it is possible to administer to the roots of broad beans certain substances which are absorbed and transferred to the leaves and stems. In experiments in which pyridine was used it is shown that this substance, in suitable concentrations, exercises a marked detrimental effect upon the aphids. There are many features which render the exact conditions rather difficult to define. The great majority of these features are concerned with the conditions governing absorption

by the plant and with the effect of the pyridine on the plant after absorption. It was noticeable in the sand experiments that the effect on the aphids was to a large extent proportional to the amount of pyridine administered to the plant. The experiment in which dry weights of treated and control plants were compared shows quite plainly that a very evident effect is caused by the pyridine. In experiments on plants growing in soil the pyridine appeared to have a much less detrimental effect on the plants. It still had a very obvious effect on the aphids when present in sufficiently high concentration. This concentration had to be much higher than was the case with plants grown in sand.

*(Received April 19th, 1929.)*



# ON THE ECONOMIC STATUS AND BIONOMICS OF *SMINTHURUS VIRIDIS*, LUBB. (COLLEMBOLA).

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(PLATE XIV.)

The inclusion of species of the order Collembola among the list of injurious insects has, from time to time, given rise to considerable scepticism among those concerned with economic entomology. It is, therefore, thought desirable to place on record controlled experiments which leave no doubt as to the depredations of one species, *Sminthurus viridis*, Lubb., of this primitive group of insects. Further, our knowledge of the bionomics of Collembola is so scanty that data obtained with reference to the life-history and habits of this particular species may be of interest.

## Geographical Distribution and Description.

*Sminthurus viridis*, Lubb. (fig. 1) is the only British species now included in the genus *Sminthurus*, of the subglobular forms (Symphypleona) of Collembola. This

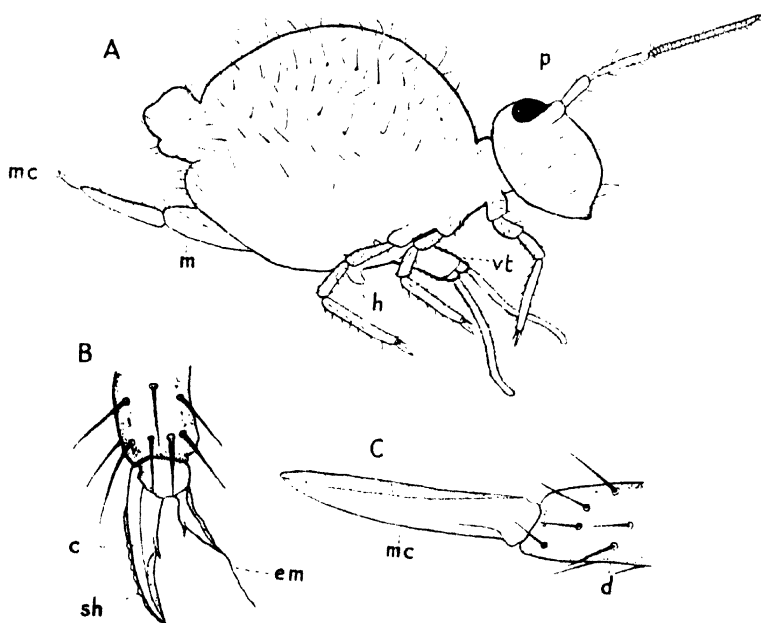


Fig. 1. *Sminthurus viridis*, Lubb.: A, adult,  $\times 40$ ; p, pigment surrounding eyes; vt, ventral tube with extended warted vesicles; h, hamula; m, mandibles of furcula; mc, mucro; B, foot of left posterior leg,  $\times 270$ ; c, tarsal claw; em, empodium; sh, membranous sheath; C, furcula,  $\times 270$ ; d, dentes; mc, mucro.

greenish yellow springtail is found commonly, especially in spring and autumn, on grassland and clover leas all over the British Isles, while its distribution abroad is very wide, it being recorded from Australia, New Zealand, and the whole Palaearctic region. The average size of adult specimens is 2.0-2.5 mm.; the head is anteriorly

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obtuse, the angle enclosing the mouth-parts, which are adapted for both biting and sucking. The eyes, situated on a patch of black pigment on the posterior half of the head, stand out in contrast to the green body. The 4-jointed antennae, slightly anterior in position to the eyes, are also green except for the terminal segment, which is reddish in colour and is marked off into a series of about 20 constrictions, each with a whorl of fine hairs around its circumference. The thoracic segments are very much reduced, being telescoped between the head and the abdomen, the latter comprising about two-thirds the size of the entire insect. The legs are five-jointed and terminate in two unequal tibio-tarsal claws (fig. 1, B).<sup>\*</sup> The abdomen, subglobular in form, bears ventrally the characteristic appendages of Collembola; the ventral tube is long and its walls are covered with warted prominences; the *hamula* or "catch" on the 3rd segment is well developed, as also is the *furcula* or "spring" with its stout *manubrium* and elongate *dentes* and *mucrones* (fig. 1, C). The whole body and legs are covered with fine hairs, and there are no glandular openings on the furcal segment.

*Sminthurus viridis* is phytophagous in its feeding habits, despite the fact that many of its related species are saprophytic. Lubbock<sup>1</sup> in 1873 stated that "some species of *Smynturus* live on leaves"; this was the first indication that Collembola may be of economic importance. *Sminthurus viridis* is omitted in Theobald's<sup>2</sup> list of injurious species of Collembola in 1910, but it is possible that some of the previous records of damage by *Sminthurus* sp. may have referred to this particular species. The first authentic record of damage by this species appears to be that of Lea<sup>3</sup> in 1920, when under the popular name of "lucerne flea" it was reported to cause considerable damage to that particular host-plant in South Australia. Subsequent reports (1921-1924) from the same area have been published, and among its host-plants are lucerne, clover, Cape dandelion, beans, potatoes, sugar-beet, oats, barley, and grasses. *Sminthurus viridis* was declared a pest under the Plant Diseases Act, 1924, in New South Wales.<sup>4</sup> It is interesting to note also that under the new scheme of the Empire Marketing Board (1927) for the distribution of parasites of insect pests to the Colonies, one of the first demands was that from Australia for parasites of this insect. In Great Britain, however, its economic significance appears to have been overlooked, and probably owing to its elusive habits, its depredations have been included among those of species of *Apion*, *Hypera*, etc.

### Experimental Evidence of Damage.

During an investigation of the economic position of Collembola the writer was able to make detailed observations regarding the occurrence and habits of *Sminthurus viridis* on clover leas at Rothamsted. On witnessing, under a hand-lens, several specimens feeding on clover leaves and realising the enormous numbers present, it became desirable to estimate, at least qualitatively, the amount of damage caused by these insects. A series of 15 flower-pots containing uniform, insect-free soil, was set up; each was covered by means of a lamp-glass (basal diameter  $3\frac{1}{2}$  inches) and, after 50 seeds of wild white clover (*Trifolium repens*, L.) had been sown in each, the top of the lamp-glass was closed by a cap consisting of a circular iron ring (5 in. diameter) covered with fine muslin. These pots were placed in the insectary, where conditions were comparable to those out of doors except that watering was necessary. The clover seeds germinated in 5 days and were allowed a further 5 days to develop before being infested with the Collembola. At this period the 10 most uniform pots of clover were selected for experimental work. The average number of *Sminthurus viridis* for that particular surface area in the field was ascertained by taking 40 counts within the lamp glass on the clover lea on Little Knott field; 6 proved to

<sup>\*</sup> Specimens mounted in Keilin's medium reveal a wavy membranous sheath (*sh*) adjacent to the outer edge of the upper claws.

be the average number of individuals per unit area. On 16th March 1926 a quantity of *Sminthurus viridis* was swept from the field and 6 adult specimens were placed in each of the 5 clover pots in the insectary; the 5 remaining being kept as controls. On the following day the clover in the infested pots showed obvious evidence of damage, and individuals of *Sminthurus viridis* were observed feeding indiscriminately on the upper and lower surfaces of the leaves. Later observations showed that frequently the outer epidermis, upper or lower as the case may have been, was left untouched, giving a skeletonising effect as seen in Plate xiv, fig. 2. There was ample evidence that feeding took place during both day and night, and under very warm dry conditions feeding and movement were confined to the base of the herbage. The effect of attack by these few individuals was soon evident and is clearly seen in the photographs (Pl. xiv, fig. 1) taken on 5th May. One control and 2 infected pots are shown and illustrate the extent of damage caused to clover by 6 individuals of *Sminthurus viridis* for a period of 10 weeks. It should be added that the pots were observed daily and the six individuals counted; in the event of death of an individual another specimen was added. The marked effect was very evident at the end of 15 weeks, when the clover, under the favourable conditions of the control pots, outgrew the height of the lamp-glasses.

This conclusive evidence, together with personal observations in the field, convince one of the damage this species may do, especially on young clover leas. The fact that *Sminthurus viridis* has been observed by the writer to feed also on turnips, mangels, barley, oats, wheat and grasses, makes it possible for this insect to exist on other crops in the rotation and thus tide over arable conditions and be present when the field is laid down to clover or grass.

Realising the fact that *Sminthurus viridis* had several host-plants, experiments were set up with a view to discovering, if possible, whether this species was selective in its feeding habits. Ten large earthenware dishes, 8 inches in diameter, were filled with insect-free soil, and 20 seeds of each of the following grasses or clovers of a common seed mixture were sown in separate rows: Wild white clover (*Trifolium repens*, L.), late-flowering red clover (*Trifolium pratense*, L.), perennial rye grass (*Lolium perenne*, L.), Italian rye grass (*Lolium italicum*, A.Br.), cocksfoot (*Dactylis glomerata*, L.), timothy (*Phleum pratense*, L.). The dishes were then covered with large glass cylinders, 7 inches in diameter, closed in above by muslin caps. When the seedlings had become fully established 20 specimens of *Sminthurus viridis* were introduced into each cage. Feeding took place more or less indiscriminately, except that the wild white clover showed evidence of greater attack in the earlier stages. The insects were allowed to feed for 14 days, after which the grasses and clover plants were examined individually. Counts of damaged plants at this stage gave no significant difference, as specimens of all the species were found to be attacked. These negative results were surprising in view of the fact that ample evidence was obtained in the field by sweeping that there was a greater preponderance of *Sminthurus viridis* on patches of clover over those areas where little clover existed. It may be, however, that the conditions of confinement in these cages prevented any selective habit being established. Specimens of *Sminthurus viridis* have been swept from grassland at Rothamsted in every month of the year, and thus the damage caused by this pest over such a period must be considerable.

### Control Measures.

It is regretted that up to the present no opportunity for investigating a practical method of control has occurred, but from general observations it is thought desirable to suggest the following methods. Sweeping of infected areas with tarred sacks, as used against another species *Bowlettiella hortensis* on mangels,<sup>6</sup> would no doubt reduce the numbers considerably, but the presence of the "nurse crop" prevents

this from being done at the critical stage in the life of the young seedling clover or grass. The extremely delicate structure of *Sminthurus viridis*, and the presence of destroyed specimens on stones and elsewhere following a heavy rolling, suggest that this operation, together with chain-harrowing would assist in reducing the numbers of springtails. By arranging tarred sacks in front of the roller, the efficiency of this method would be increased. In this connection it may be of advantage to note that Spafford<sup>6</sup> recommends close grazing by sheep and chain-harrowing as a means of reducing the numbers of the lucerne flea in New South Wales. In exceptional cases, such as greenhouse attack, or heavy local infestation, dusting with 5 per cent. nicotine sulphate has afforded a control.<sup>7</sup> No insect parasites have, as yet, been bred from Collembola, so that it remains for predatory enemies to keep these insects in check. The chief of these, so far as the writer is aware, is a small black spider (unidentified as yet). The scarcity of *Sminthurus viridis* on Little Knott field at Rothamsted, when these spiders were present, was very evident; its sudden method of approach is extremely efficient in capturing its prey. The remark by several farmers that black spiders were eating their clover is no doubt associated with this fact.

### Bionomics.

Apart from the fact that Collembola undergo no metamorphosis, little is known regarding the life-history of this primitive group of insects. During the present



Fig. 2. A typical batch of eggs of *Sminthurus viridis* laid on damp decayed leaves.  $\times 15$ .

investigation many interesting observations have been made on the habits of *Sminthurus viridis*; these are included here as a preliminary account, as it is hoped to carry out a more detailed study of this subject later.

As previously stated, specimens of *Sminthurus viridis* have been collected in the field during each month of the year. Specimens caught in the field were periodically brought into the insectary, where they were placed on clover leaves in small glass dishes, lined with filter-paper which was kept continually damp. Under these

conditions eggs were easily secured. Oviposition was observed each month from March 1926 to November 1926, observations having been unavoidably suspended for the remaining months. The eggs (fig. 2) are pale yellow in colour, with smooth delicate chorion, and 0.25 mm. in diameter at the time of oviposition. During oviposition the female stands with legs spread well apart and lowers its head until it touches the filter-paper, its antennae being held in a dorso-posterior position. A yellow substance slowly issues from its anal end, which is raised; the mass being originally without definite shape gradually assumes a spherical appearance. With the yellow globule protruding the female raises its head and lowers its anal end until the spherical mass touches the filter paper. The mass, or egg as it proves to be, adheres to the surface, and the female after vigorous movement eventually releases itself. During this process the egg, and subsequently a batch of eggs, become covered with excreta issuing from the anus, which in some cases completely hide the eggs. This, no doubt, accounts for the fact that it is extremely difficult to discover eggs in the field. After oviposition the female turns round and touches the egg with its antennae and feet. The eggs are laid in batches varying in numbers from 2 or 3 to 30 or 40, according as to whether the female is disturbed or not during oviposition. Eggs are usually laid on damp decaying leaves and other moist surfaces at the base of herbage; occasionally they were found singly on the underside of the lower clover leaves.

The dishes in the laboratory were retained at an average temperature of 15° C., and damp conditions were maintained continuously. The average diameter of the egg at oviposition is 0.25 mm. The diameter gradually increases, and when at 0.27 mm., 20–25 days after oviposition, the eyes and antennae are visible through the delicate chorion. Shortly after this stage an equatorial split occurs, which widens as the two halves of the chorion are gradually forced apart. Under the conditions mentioned, hatching took place between 35 and 40 days after oviposition. Emergence is effected by the anterior half of the chorion being pushed off by the head of the individual, and frequently vigorous movement is necessary before the insect frees itself from the egg. The spring, or furcula, is at first fully extended, but immediately the individual is free, it retracts its furcula under its abdomen, after which it is capable of movement by leaping. Its size on emergence is 0.4 mm. Specimens have been observed feeding a day after emergence. Gradual growth takes place until the individual becomes mature in about 8–10 weeks, according to atmospheric conditions. No mating was observed, nor has any ever been recorded among Collembola. It is hoped that further observations may yield information on this matter.

Whereas in all elongate species (Arthropleona) of Collembola cast skins are exceptionally common among the haunts of these species, none were observed in the case of *Sminthurus viridis*. Despite lengthy observations with a view to witnessing this phenomenon, no indication of moulting was seen.

The writer is grateful to Dr. A. D. Imms for the valuable advice and facilities rendered during this investigation.

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#### References.

1. LUBBOCK, J. (1873). Monograph of the Collembola and Thysanura.—Ray Society.
2. THEOBALD, F. V. (1910). Springtails (Collembola). Their Economic Importance with Notes on some unrecorded instances of Damage.—1er Congrès Internat. d'Entom. (Bruxelles), ii, p. 1–18.

- 3(a). LEA, A. M. (1920). Lucerne Flea.—Jl. Dept. Agric. S. Australia, Adelaide, xxiii, no. 6, p. 502.
- (b). LEA, A. M. (1922). Lucerne Flea.—Jl. Dept. Agric. S. Australia, Adelaide, xxvi, no. 5, pp. 423–426.
4. (1925.) Proclamation under the Plant Diseases Act.—Govt. Gaz. no. 97, Sydney, 17th July 1925.
5. DAVIES, W. M. (1926). Collembola injuring leaves of Mangold seedlings.—Bull. Ent. Res., xvii, pp. 159–162.
6. SPAFFORD, W. J. (1921). Lucerne Flea.—Jl. Agric. S. Australia, xxiv, no. 11, p. 893.
7. DAVIES, W. M. (1925). Springtails attacking Mangolds.—Journ. Ministry Agric., xxxii, no. 4, pp. 350–354.

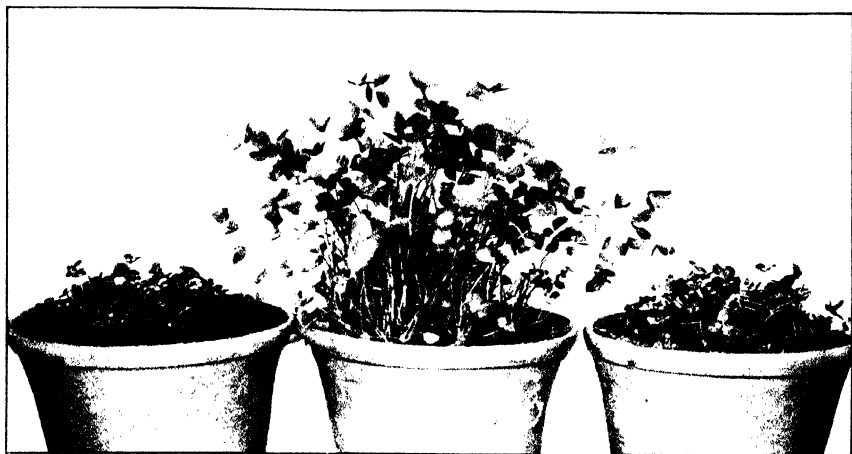


Fig. 1. Experimental pots containing 50 plants of wild white clover, showing the damage caused by six specimens of *Sminthurus viridis* in ten weeks; the middle pot is the control.

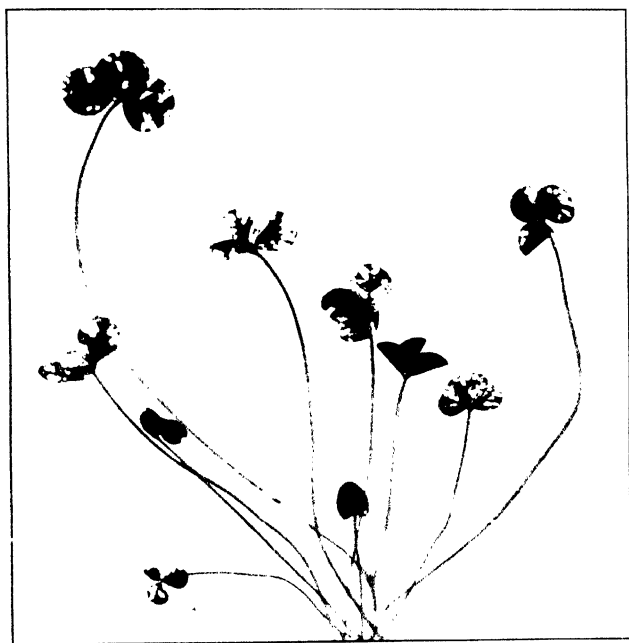


Fig. 2. Leaves of wild white clover (*Trifolium repens*) showing damage under controlled conditions caused by *Sminthurus viridis*, Lubb.



# THE EFFECT OF VARIATION IN RELATIVE HUMIDITY ON CERTAIN SPECIES OF COLLEMBOLA

BY W. MALDWYN DAVIES, B.Sc., PH.D.<sup>1</sup>

(From the Entomology Department, Rothamsted Experimental Station.)

(Received 6th June 1928.)

## 1. INTRODUCTION.

THE present work was commenced in order to ascertain the effect of humidity on certain species of Collembola and, because it was felt that before any critical work of the study of chemotropism could be undertaken with Collembola, a knowledge of the behaviour of particular species in respect to humidity was essential.

Conflicting statements have been made regarding the optimum condition of humidity associated with the presence of Collembola. Curtis (1860) stated that "these ground-fleas will not remain on damp ground, they may be expelled by sprinkling salt over the land." The work of Davenport (1903), on the other hand, suggests that for particular species, viz. *Anurida maritima* Guerin, *Xenylla humicola* Tull. and *Isotoma besselsii* Pack., the contrary is the case. These species he remarks, are "exceedingly sensitive to variation of atmospheric moisture" with which they are in almost continued contact.

It was found that certain limitations were inevitable if the work was to be of a comparative nature. Firstly, it would be necessary to confine the experiments to species which could be easily identified and could be secured in sufficient numbers to yield significant results. The species used were: *Sminthurus viridis* Lubbock, *Dicyrtomina minuta* Fab., *Entomobrya multifasciata* Linn., *Isotoma viridis* Bourl. and *Tomocerus vulgaris* Tullb. Secondly, it was essential for comparative results to secure the species locally and to be able to transfer them quickly from their natural habitat into the experimental chamber. To eliminate any effect due to change in humidity during transit to the laboratory and to ensure that all individuals possessed an equal degree of humidity before the experiment, all the specimens were transferred to the surface of water before being introduced into the experimental containers.

## 2. TECHNIQUE.

In carrying out the studies on relative humidity the temperature was kept constant by using the constant temperature room at Rothamsted, where heating is maintained by means of a gas radiator regulated by a thermostat. Since the experiments had to continue throughout the year the minimum constant tempera-

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ture which could be used was, of course, influenced by the maximum outdoor temperature in summer and it was decided that 25° C. would be the most favourable. The variation in humidity was obtained by varying the strength of sulphuric acid in a series of 2000 c.c. flasks, thus giving the relative humidity as indicated by Regnalt's table. Pure sulphuric acid was used and the absence of any injurious effect of the acid was proved by a control experiment being made with calcium chloride as a dehydrating agent, when comparative results were obtained. The presence of any irritating substance is, however, quickly detected by *Collembola* which jump violently when in contact with it; in these experiments the *Collembola* moved about quite normally. Furthermore, specimens which had been in the dry atmosphere until moribund, when removed and placed on water, gradually lost their shrivelled appearance and became quite normal and active. Six 2000 c.c. flasks were set up with the following relative humidities: 0, 10, 20, 50, 90 and 100 per cent. Suspended by means of a tape, from the rubber bung of each flask, was a short glass tube of 1½ in. diameter, closed below by a piece of cheese cloth and, above, by a cork. The humidity in each flask was allowed to attain equilibrium over night. As previously mentioned the specimens of *Collembola* were collected in the field, transferred on to water in a dish in the constant temperature room, and then 10 individuals were placed in the small glass tube which had been quickly removed with the bung and quickly replaced, with its contents, into the flask. Preliminary experiments yielded data giving the period of time necessary for periodic observation and, as will be seen, this varied for different humidities and different species from 1 minute to half-hour intervals. From these observations the death-rate of individual species in different humidities was ascertained. Usually the number of deaths could be observed through the flask but occasionally it was necessary quickly to remove the container, count the number of dead specimens present and replace. Five experiments each with 10 individuals were carried out for each humidity test.

### 3. THE EXPERIMENTS.

#### *Isotoma viridis* Bourl.

This is one of the most widespread species of *Collembola* and is usually found in damp situations, being abundant on most soils. It is one of the *Arthropleona* forms, its body length measuring 2–3 mm. Its respiratory system is cutaneous and it possesses a small ventral tube. The behaviour of *Isotoma viridis* in varying humidities is seen in Table I.

It is seen that *Isotoma viridis* is extremely susceptible to changes in atmospheric moisture, for it is unable to survive longer than 20 minutes in the driest conditions. This insect, like the other species of *Collembola*, does not appear to react differentially to the lower degrees of humidity, for the death-rate in the 20 per cent. flask is practically the same as in those of 0 and 10 per cent. There is a slightly slower death-rate in the 50 and 90 per cent. relative humidities, while there is a striking contrast in the 100 per cent. flask. An interesting point arises, where it

is possible to maintain a saturated atmosphere without the deposition of water. Under these conditions the cheese cloth, forming the floor of the container, does not bear excess water on its surface and it was found that when *Isotoma viridis* was introduced into a flask with such atmospheric conditions, the death-rate closely approximated to that of the 90 per cent. relative humidity. On the other hand, when deposition of water does take place the death-rate is as shown under the column for the 100 per cent. relative humidity. Generally speaking it would then appear that Collembola (for this result occurred with the other species except *Entomobrya multifasciata* used in these experiments) need to be in actual contact with the surface film before they can absorb moisture. If it had been physically possible constantly to obtain conditions of a saturated moist atmosphere with a dry surface on the cloth, a valuable comparison could have been made but, of course, a slight fluctuation in temperature immediately resulted in a deposition of moisture on the glass and cloth. The 90 per cent. relative humidity flask then will have to be considered as the dry cloth in an almost saturated atmosphere, and the 100 per cent. flask as the wet cloth in the completely saturated atmosphere. It will be noticed throughout, except perhaps where a starvation factor comes in, that there is a marked difference between results in the 90 and the 100 per cent. relative humidities. The above mentioned facts possibly offer the explanation for this.

Table I.

Percentage relative humidity	Percentage death-rate of <i>Isotoma viridis</i> in varying degrees of relative humidity																			
	Time in min.				Time in hours															
	5	10	15	20	$\frac{1}{2}$	$\frac{3}{4}$	1	$1\frac{1}{4}$	$1\frac{1}{2}$	$1\frac{3}{4}$	2	$2\frac{1}{2}$	3	$3\frac{1}{2}$	4	$4\frac{1}{2}$	5	6	7	8
0	4	46	90	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10	38	56	92	94	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
20	44	64	82	88	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
50	0	8	26	42	71	98	100	—	—	—	—	—	—	—	—	—	—	—	—	—
90	0	0	0	2	20	46	78	98	100	0	18	22	26	38	50	52	62	84	94	100
100	0	0	0	0	0	0	0	0	0	0	18	22	26	38	50	52	62	84	94	100

*Tomocerus vulgaris* Tullb.

This is another member of the Arthropleona which is widespread in range and usually found under damp bark and decaying vegetable matter. It measures 3-4 mm. and its body is densely covered with scales; its respiration is cutaneous and the ventral tube is slightly longer than that of *I. viridis*. The behaviour of this species in different degrees of humidity is shown in Table II.

In the lower humidities it is observed that *T. vulgaris* behaves in a similar manner to *I. viridis* but there is a marked difference between the death-rate of *T. vulgaris* in the 20 and the 50 per cent. relative humidity. It would appear that *Tomocerus* can make use of a medium degree of humidity. When we consider the higher degrees, 90 and 100 per cent., this reaction is even more evident for there is very little difference between the death-rates under either condition especially

as the starvation factor would come into operation after 24 hours. Thus it is seen that by some means or other *T. vulgaris* can make use of atmospheric moisture before it actually attains saturation point. The presence of scales in this species as compared with *I. viridis* may afford the explanation.

Table II.

Percentage relative humidity	Percentage death-rate of <i>Tomocerus vulgaris</i> in varying degrees of relative humidity																						
	Time in min.				Time in hours																		
	5	10	15	20	$\frac{1}{2}$	$\frac{3}{4}$	1	1 $\frac{1}{4}$	1 $\frac{1}{2}$	1 $\frac{3}{4}$	2	2 $\frac{1}{4}$	3	5	10	15	20	25	30	35	40		
0	2	34	84	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
10	2	30	68	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
20	4	24	58	86	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
50	0	0	0	0	4	12	22	31	46	62	88	96	100	—	—	—	—	—	—	—	—		
90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	20	96	100	—	—		
100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	98	100	—		

*Entomobrya multifasciata* Tullb.

Species of this genus are usually associated with drier surroundings than is the case with the majority of species of Collembola. *Entomobrya albocincta*, for instance, is usually found among very dry decaying material such as bark. It is regretted that this species was not available locally in sufficient quantities to use in these experiments. The entire body of *Entomobrya* is thickly covered with hairs and members of this genus possess a well-developed ventral tube. The results of experiments with the above species are shown in Table III.

Table III.

Percentage relative humidity	Percentage death-rate of <i>Entomobrya multifasciata</i> in varying degrees of relative humidity																			
	Time in hours																			
	1	2	3	4	5	6	7	10	15	20	30	40	50	60	70	80	90	100	120	180
0	0	4	20	63	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10	0	0	41	72	96	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—
20	0	0	0	20	73	92	100	—	—	—	—	—	—	—	—	—	—	—	—	—
50	0	0	0	0	0	0	20	52	100	—	—	—	—	—	—	—	—	—	—	—
90	0	0	0	0	0	0	0	0	0	0	0	0	10	24	68	82	100	—	—	—
100	0	0	0	0	0	0	0	0	0	0	0	0	0	36	48	62	70	94	96	100

It is observed that *Entomobrya multifasciata* is not susceptible to dry conditions as other members of the Arthropodea, for even in the 20 per cent. relative humidity it can exist for 6 hours while in the 50 per cent. it can live as long as 15 hours. There is, however, the same considerable difference between the death-rate in the lower and that in the higher humidities. The starvation factor no doubt brings the results of the higher humidities into close approximation. *Entomobrya multifasciata* does not require a deposition of moisture before it can avail itself of water.

The two remaining species used in these experiments are members of the group Symphypleona which includes all the subglobular forms.

*Dicyrtomina minuta* Fab.

This species is common in Great Britain where it occurs on the grass, beneath trees, and among the damp decaying leaves; it is very common in the autumn. Its subglobular surface is smooth and shiny and a definite respiratory system is absent; respiration being cutaneous. The ventral tube in this species is well developed and its walls are smooth. The death-rate of this species in varying humidities is shown in Table IV.

Table IV.

Percentage relative humidity	Percentage death-rate of <i>Dicyrtomina minuta</i> in varying degrees of relative humidity																	
	Time in hours																	
	½	1	2	1	1½	1½	2	3	4	5	10	15	20	25	30	40	45	50
0	8	48	84	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10	0	62	98	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—
20	0	38	78	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—
50	0	24	70	96	100	—	—	—	—	—	—	—	—	—	—	—	—	—
90	0	0	0	0	0	0	4	4	28	93	100	—	—	—	—	—	—	—
100	0	0	0	0	0	0	0	0	0	0	0	0	2	32	46	64	92	100

*Dicyrtomina minuta* is quite unable to withstand the dry conditions despite the possession of a well-developed ventral tube. In the moist atmosphere it is able to exist normally until, of course, the starvation factor comes into play after about 24 hours. There is, however, a very marked difference between its death-rate under almost saturated conditions with the dry cloth (*i.e.* the 90 per cent. flask) and that under the saturated conditions with wet cloth in the 100 per cent. relative humidity.

*Sminthurus viridis* Lubbock.

This species—a member of the group Symphypleona—is widespread in distribution and is very common on clover and grassland, being also a pest of lucerne in Australia. It differs from all previously mentioned species in that it possesses a well-developed tracheal system and a highly developed ventral tube provided with long protrusible vesicles. It is similar in shape and size to *Dicyrtomina minuta*, 2-3 mm.

Table V.

Percentage relative humidity	Percentage death-rate of <i>Sminthurus viridis</i> in varying degrees of relative humidity																		
	Time in hours																		
	1	5	10	15	20	25	30	35	40	45	50	60	65	70	80	90	100	150	192
0	0	54	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10	0	28	96	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
20	0	30	82	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
50	0	0	0	14	46	84	100	—	—	—	—	—	—	—	—	—	—	—	—
90	0	0	0	6	8	18	34	38	62	68	78	96	100	—	—	—	—	—	—
100	0	0	0	0	0	0	0	0	0	4	8	30	30	32	42	52	63	96	100

Examination of the above table shows that this species is well adapted to withstand dry conditions, for it can live as long as 15 hours in the lower degrees of relative humidity. Further, with a medium amount of moisture, that is, in the 50 per cent. flask, this species can exist for as long as 35 hours, when doubtless starvation accounts to a certain degree for the death-rate. In the 90 per cent. relative humidity, which has been considered as an almost saturated atmosphere, but in contact with a dry cloth, we find that *Sminthurus viridis* can live for as long as 65 hours. In the saturated atmosphere and in contact with a damp cloth, that is the 100 per cent. flask, this species lives for 192 hours when, obviously, death is due to starvation. *Sminthurus viridis*, then, is far better adapted for withstanding dry conditions, and although it remains to investigate the behaviour of many other species, before a definite correlation can be established, it does appear strongly to suggest that the presence of a tracheal system and well-developed ventral tube considerably aid in reducing susceptibility to the influence of dry conditions.

In this connection it is interesting to note that the majority of aquatic forms are cutaneous in their mode of respiration, and further, that the ventral tube in aquatic forms is considerably reduced, being a mere prominence in *Podura aquatica*.

A comparison of the death-rate of the different species in the varying humidities may perhaps be best gleaned by compiling the results as seen in Table VI and Table VII.

Table VI.

Species	Percentage death-rate of different species in 0 per cent. relative humidity															
	Time in min.						Time in hours									
	5	10	15	20	40	45	1	2	3	4	5	6	7	8	9	10
<i>I. viridis</i>	4	46	90	100	—	—	—	—	—	—	—	—	—	—	—	—
<i>T. vulgaris</i>	2	34	84	100	—	—	—	—	—	—	—	—	—	—	—	—
<i>E. multifasciata</i>	0	0	0	0	0	0	0	4	20	63	100	—	—	—	—	—
<i>D. minuta</i>	0	0	8	20	48	84	100	—	—	—	—	—	—	—	—	—
<i>S. viridis</i>	0	0	0	0	0	0	0	0	8	40	54	60	86	90	96	100

The two Arthropleona forms *I. viridis* and *T. vulgaris* succumb at approximately the same rate in the dry atmosphere (Table VI). In both, the mode of respiration is cutaneous and the presence of scales in *T. vulgaris* does not appear to assist the species in enduring dry conditions. In *E. multifasciata* it is surprising to find that this member of Arthropleona, with a cutaneous respiratory system, provides such a marked contrast to the other members of its group. It is true that all members of the Entomobrya are associated with dry conditions, and, although the ventral tube is decidedly longer in members of this genus, it is felt that some physiological explanation may provide the solution. *D. minuta*, though possessing a well-developed ventral tube, has no tracheal system, and we find that its susceptibility to dry conditions is comparatively similar to that of the Arthropleona forms, *I. viridis* and *T. vulgaris*. *S. viridis*, possessing both well-developed ventral tube and tracheal

system, affords a striking contrast in comparison, for even in this arid condition it can live for 10 hours.

The other interesting comparison is that of the different species in 100 per cent. relative humidity and the compiled results are shown in Table VII.

Table VII.

Species	Percentage death-rate of different species in 100 per cent. relative humidity																	
	Time in hours																	
	1	5	8	10	20	25	30	40	45	50	60	70	80	90	100	120	150	192
<i>I. viridis</i>	0	62	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>T. vulgaris</i>	0	0	0	0	10	16	98	100	—	—	—	—	—	—	—	—	—	—
<i>E. multifasciata</i>	0	0	0	0	0	0	0	0	0	0	36	48	62	70	94	96	100	—
<i>D. minuta</i>	0	0	0	0	2	32	46	64	92	100	—	—	—	—	—	—	—	—
<i>S. viridis</i>	0	0	0	0	0	0	0	0	4	8	30	32	42	52	63	80	96	100

Here the time of duration has been removed from minutes to hours and *I. viridis* exists as long as 8 hours, under the saturated conditions of this experiment. There is a definite gradation to the case of *T. vulgaris* where it remains as long as 40 hours. It is suggestive that the presence of scales may facilitate this species in making use of the moist atmosphere.

*E. multifasciata* again affords a contrast in that no insects die until after the 50-hour period and some remain alive as long as 150 hours. *D. minuta* with its lack of tracheal system appears to find conditions quite favourable in a saturated atmosphere, for death does not occur until after the starvation factor begins to operate.

Similarly *S. viridis* is only influenced by starvation in the 100 per cent. relative humidity, for it can exist as long as 190 hours when obviously the insects died from starvation.

An interesting case of the association of moisture and the function of the ventral tube, was brought to light by means of the following experiment. Specimens of *S. viridis* were taken after several hours' exposure to the 0 per cent. relative humidity and these were finely sprayed with water. Observations were then made under the binocular microscope. It was seen that the specimens shot out the vesicles of their ventral tubes over the dorsal region. This caused the droplets of water on the hairs to be removed. The movement was so rapid that it was impossible to see what became of the droplets. However, the experiment was elaborated by first spraying with water, then dusting with fine glucose, and the observations continued. The vesicles of the ventral tube were again shot out, and this time the granules of glucose adhered to them, then the vesicles were withdrawn and the process this time was hindered by the presence of the granules, for it was clearly seen that the vesicles were drawn across the mouth and the granules thus removed. Thus the ventral tube serves as a cleaning organ, but it appears that, in this instance, it primarily served for removing droplets of moisture from the surface of the body to the mouth, and thus has a direct relation to the assimilation of moisture.

The susceptibility of Collembola to atmospheric moisture has proved to have a direct practical application. During an attack on mangolds by *Bourletiella hortensis* in June 1925 a method of control was sought. Trapping on a tarred surface afforded an efficient control (Davies, 1926) but it proved essential to the success of the control that the method should be applied in the early morning or after rain had fallen, for then the Collembola were present in large numbers. When the surface became dry the springtails moved down into the soil nearer the damper conditions.

The writer is greatly indebted to Dr Imms for his valuable advice in this work and also to Mr G. O. Lunnion and Mr H. C. F. Newton, for assisting in the observations and in recording the death-rate in some of the experiments.

#### 4. SUMMARY.

1. Authorities have differed regarding the optimum degree of moisture associated with the presence of Collembola. Experiments have, therefore, been carried out to ascertain the influence of variation in relative humidity on the death-rate of certain species of these insects.

2. At a uniform temperature of 25° C. it was found that saturated conditions of the environment are necessary for the survival of all species of Collembola used in the experiments.

3. It has been found that, with the exception of the genus *Entomobrya*, Collembola devoid of a tracheal system are very susceptible to dry conditions. *Sminthurus viridis*, which possesses a tracheal system and well-developed ventral tube, is much less susceptible to atmospheric dryness.

4. An experiment carried out with *Sminthurus viridis* suggests that the ventral tube is used for conveying droplets of water from the hairs of the body to the mouth. It also serves as a "cleaning" organ.

5. The results of the above experiments have had direct practical application in methods of control of Collembola.

#### 5. LITERATURE CITED.

- CURTIS, J. (1860). *Farm Insects*, p. 433.  
DAVENPORT, C. B. (1903). "The Collembola of Cold Spring Beach with special reference to the movements of the Poduridae." *Cold Spring Harbour Monographs*, 2.  
DAVIES, W. M. (1926). "Collembola injuring leaves of mangold seedlings." *Bull. Ent. Research*, 17, Pt. 2, 159-162.

# THE BIONOMICS OF *APION ULICIS* FÖRST. (GORSE WEEVIL), WITH SPECIAL REFERENCE TO ITS RÔLE IN THE CONTROL OF *ULEX EUROPAEUS* IN NEW ZEALAND<sup>1</sup>

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(With Plates XV-XVII and 3 Text-figures.)

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## 1. INTRODUCTION.

BIOLOGICAL control has, during recent years, made considerable advance in its position in economic entomology. This has resulted chiefly from the study and application of biological control of insect pests. Latterly, however, a further aspect of this subject has been considered, namely, the biological control of noxious weeds; it is with this section that the present paper is concerned.

The works of Perkins and Swezey<sup>(15)</sup>, Koebele<sup>(14)</sup>, Alexander<sup>(1)</sup>, Imms<sup>(12)</sup> and Tillyard<sup>(19)</sup> have dealt exhaustively with the present position of this subject, so that a brief summary will here suffice.

It is obvious that the introduction of insects into a country as a means of controlling a noxious plant, can only be adopted when the

<sup>1</sup> Part of thesis approved for the Degree of Doctor of Philosophy in the University of London.

nature of the problem is sufficiently grave as to necessitate an attempt being made with every possible safeguard, and under most critical scientific supervision.

The pioneer work in the control of noxious weeds was done in the Hawaiian Islands, where the colonisation of a number of species of insects introduced from Mexico afforded an appreciable control of the injurious plant *Lantana camara*. Investigations regarding the repression of nut-grass (*Cyperus rotundatus*) by the introduction of insect enemies from the Philippines, has also been the subject of experiment in the Hawaiian Islands.

The most formidable attempt at the control of noxious weeds by means of insects is that of the Prickly Pear campaign in Queensland and New South Wales. So serious became the spread of the introduced plant, that in 1919 a "Commonwealth Prickly Pear Board" was established to deal with the problem. Insect enemies of the Prickly Pear were searched for in the warmer parts of America, and the first consignment shipped to Australia in March 1921. The outcome of this, and later, importations is that Cochineal insects of the genus *Dactylopius* have already destroyed large areas of this noxious weed. Recent reports from the areas where this work of weed control is being carried out are extremely encouraging.

A great stimulus to this new line of work has been provided by the scheme that has been financed by the Empire Marketing Board in conjunction with the New Zealand Government and the Cawthron Institute, Nelson, to ascertain the possibilities of establishing a control of noxious weeds in New Zealand by the introduction into that country of appropriate insects. A proportion of the above grant has been allocated by the Cawthron Institute to the Rothamsted Experimental Station, and it is with the financial assistance thus available that the experiments involved in the present paper have been carried out. The work has been prosecuted under the direction of Dr A. D. Imms whose valuable advice is gratefully acknowledged. The writer is also greatly indebted to Dr R. J. Tillyard who instigated the work in New Zealand.

*Ulex europaeus* L. (common gorse or furze) is included among the plants scheduled by the New Zealand Government<sup>(21)</sup> as noxious weeds. According to Thomson<sup>(18)</sup> it must have been introduced at an early date, as it is noted that Darwin observed plants of gorse in 1835. Since its introduction it has spread very rapidly and now covers large areas of ground, threatening to render derelict some of the most valuable pasture land of that country. A similar occurrence is in the Hawaiian

Islands, where it is stated that 17 years ago the first and single plant of *Ulex europaeus* was observed. Subsequently this plant has spread at an extraordinary rate, and the possibilities of devastation of valuable land has been so keenly felt that it has been proposed that large sums of money be spent to attempt to eradicate this weed. The methods used are chiefly mechanical, gangs of men being employed to dig out and burn the young seedlings. That the plant spreads by means of its seed has been fully proved, owing to the fact that young seedlings are found many miles away from the original plant.

In the young stage *Ulex europaeus* is used for sheep grazing and, further, being a leguminous plant it is highly beneficial in unmanured areas. The problem then is one of *control* rather than *eradication*. An insect which is effective in destroying the seeds will considerably assist in solving the problem. As will be seen later, a survey of the damage caused by *Apion ulicis* to gorse seeds in Great Britain has been made, and it is evident that this insect should receive full consideration.

## 2. SYNONYMY OF *APION ULICIS* FÖRST.

*Apion ulicis* Först. is the name under which it is most commonly known; it was originally described by Förster(6). It was described by Fabricius(5) as *A. nigrirostre* and again in 1808 by Kirkby as *A. ilicis*. Gyllenhal(10) named it *A. carpini*, while in 1882 Gredler described it under the name of *A. sarothamni*. Schilsky(17) considered it a variety, *nigripes*.

## 3. DESCRIPTION OF ADULT (Fig. 1).

The generic characters, viz. pear-shaped body with long, slender curved rostrum, together with long trochanters and straight antennae, are well marked. The *body* is convex, with integument rugosely punctured and pitchy black in colour, but with the exception of the eyes, rostrum, antennae, scutellum and joints of the legs, it is densely squamose, the thick white scales giving it a characteristic grey colour. *Head*, short, with the black convex eyes moderately separated; the black rostrum, shorter in the male than in the female, is narrow and slightly curved. The *antennae*, sparsely covered with fine white hairs, are straight, slender, clubbed, and arise from the base of the rostrum, the point of insertion being marked by a strong black chitinous projection. The *thorax*, practically equal in length and breadth, is slightly narrower in front, rounded behind the middle and contracted at the base; the scales are more or less irregularly scattered but are wanting from a longitudinal area in front of the scutellum. The *scutellum* is smooth and black. The

*elytra* are convex, and have the scales arranged in the form of longitudinal striae, the second of which is united at the apex to the eighth. The *legs* are black, anterior pair are sometimes reddish, and except for the joints, they are covered with scales; they are comparatively long with well-developed coxae; femora slightly dilated at the apex; tibiae more or less straight with long tarsi terminating in a bifid claw. The *abdomen* is covered with scales ventrally, these being absent at the junction of the segments. Size 2-2.75 mm.

### *Sexual Differentiation.*

Generally speaking, the male is slightly smaller than the female, but the sexes are easily distinguished by the length of the rostrum, the proportions of that of the male to that of the female being 7 : 12. The antennae are proportionately shorter in the male. No apparent difference in the abdominal segments occurs.

### *Description of Mouth Parts of the Adult.*

*Dorsal view* (Fig. 2 a). The mouth parts of the male are similar to those of the female; those of the female are described. The elongate nature of the rostrum results in a modification of the normal mouth parts. The *labrum* and *clypeus* are not present and the *epistoma*<sup>1</sup> (*epi*) is merely indicated by a faint line which divides off the apex of the rostrum. The *mandibles* (Fig. 2 b) are well developed and tri-dentate in form, the apical tooth is the largest, and the lateral one curves slightly dorsally. In structure the mandible and its attachments closely resemble that of *Pissodes strobi*, as figured by Hopkins (11). The ventral articulation has a median "ball" condyle (*c*) surrounded by a deep fossa—the *ginglymus* (*gm*). The abductor (*ab m*) and adductor (*ad m*) muscles are attached to the sides of the fossa. The so-named *pharyngeal bracon* (*ph b*) of Hopkins is present and has its surface covered with papillae. This structure extends into the pharynx, and the fact that the papillae point posteriorly suggest that the organ functions along with the ligula and lacinia in facilitating the passage of the food within the elongate rostrum.

*Ventral view* (Fig. 3). The ventral side of the rostrum is entirely complete, there being no hypostomal punctures of any kind on this surface. The *maxillae* (*mx*) are well developed except that the *cardo* (*cd*), *subgalca* (*sg*) and *stipes* (*st*) are ill-defined, being represented by one broad lobe without sutures. The *palpifer* (*f*) is large and bears a stout 2-jointed *maxillary palpus* (*mx p*), the apex of which is fringed with

<sup>1</sup> The terminology here used is that of Hopkins (11).

tubercles and the base of the terminal segment possesses strong spines. The *lacinia* (*lc*) is also well developed and covered with papillae. The *labrum* consists of an elongate *submentum* (*sm*) with its apex more or less rounded supporting the *mentum* (*mt*) which is as long as the submentum. The mentum has two strong elongate spines slightly posterior to its middle line and anteriorly bears two unsegmented *labial palpi* (*lp*), which also possess strong hairs. There is a well-developed undivided *ligula* (*lg*) the surface of which is covered with dense papillae.

### *Reproductive Organs.*

No detailed study of development has been undertaken, but reproductive organs of both sexes have been examined periodically from the time of emergence from the pod in the Autumn to the period of mating in the Spring. When the weevils emerge from the pod the reproductive organs are exceedingly immature, the ovaries being merely fine tubules. It is interesting to note further, that if for some reason or other, the pod does not open until Spring—one instance in May 1927 was noticed—the reproductive organs of these imprisoned weevils still remain immature and do not begin development until after the weevil has escaped and commenced feeding. This probably accounts for the fact that oviposition is spread over a comparatively long period. Normally the reproductive organs are mature about February or March.

### *Male Reproductive Organs.*

The male reproductive organs are shown in Fig. 4.

The *testes* (*t*) are bifollicular, each follicle being globular and of equal size, white in colour and, when mature, measures 0.23 mm. in diameter. The paired *vasa deferentia* (*vd*) are comparatively short and immediately after leaving the testes become slightly swollen, this region probably being that of the *vesicula seminalis* (*vs*). Well developed *accessory glands* (*ag*) measuring 0.8 mm. in length are present; these also arise early along the course of the *vasa deferentia* and are swollen at their apices. The *ejaculatory duct* (*ed*) follows from the junction of the paired *vasa deferentia* and leads into the *transfer apparatus* (*tr*) where it is surrounded by the strongly chitinous walls of the latter. The main section of the chitinous apparatus gives rise anteriorly to two chitinous rods 0.6 mm. long, arranged in a U-shape. The larger portion mentioned is 0.8 mm. long and 0.08 mm. broad at the point where the arms join it; it is slightly curved and terminates in a fine point surrounding the *aedaegus* (*a*). There is also an additional, more or less straight, chitinous rod (*r*)

measuring 0.4 mm. in length, which serves as a further support of the transfer apparatus. Strong longitudinal muscles (*m*) are attached to the proximal end of this rod and also to the proximal and distal end of the entire transfer apparatus. These muscles serve in extending the apparatus during copulation. There is no indication of claspers.

#### *Female Reproductive Organs.*

The female reproductive organs are shown in Fig. 5 and measure from the vaginal opening to the terminal filament from 2.0–2.5 mm.

The *terminal filaments* (*t*) are exceedingly slender and very easily separated. There are four *ovarioles* (*o*) measuring 1–1.5 mm. in length and 0.1–0.12 mm. in maximum breadth. The presence of eggs in the *vitellarium* (*vit*) is easily detected in mature specimens. The ovarioles unite to form the *oviducts* (*od*) which are short, measuring 0.05–0.8 mm. These lead into the common duct or *uterus* which measures 0.5–0.7 mm. in length and at its anterior end is 0.12 mm. in breadth. The uterus terminates in the *vagina* (*vg*) which is slightly wider and is protected posteriorly by chitinous sclerites. The vagina gives rise dorsally to a pouch-like *bursa copulatrix* (*b c*) of 0.42 mm. length and 0.15 mm. breadth; it is slightly curved at its distal end. The *spermatheca* (*sp*) is strongly chitinised and curved in form; it unites with the uterus by means of a fine *spermatic duct* (*sp d*). There is a small spherical *accessory gland* adjacent to the spermatheca. The long chitinous rod (*r*) of the ovipositor measures 0.8 mm. in length, it is swollen at its apex, and strong longitudinal muscles (*m*) are here attached. Two shorter chitinous spicules are present at its base. There is no indication of an egg calyx commonly found in Rhyncophora.

#### 4. THE EGG (see Fig. 13).

The egg is smooth with delicate yellow chorion. At the time of oviposition it is elongate in shape measuring  $0.4 \times 0.2$  mm., later it assumes a glossy white appearance and becomes more round in shape measuring  $0.35 \times 0.25$  mm.

#### 5. THE LARVA (Fig. 6).

The larva is typical of the Curculionidae being eruciform and apodous. It is a yellowish white, fleshy grub, and on emergence from the egg measures 0.5–0.6 mm. long by 0.25 mm. in width. At maturity the larva is very plump and practically incapable of movement; its measurements are as follows: length of body, including head capsule 2.5 mm., breadth

in abdominal region 1.3 mm., head capsule 0.15 mm. long and 0.16 mm. broad just behind the middle line. The entire body is strongly crescentic and sparsely covered with fine hairs.

### Head.

*Dorsal view* (Fig. 7). The head is well developed, testaceous in colour in the early stages but becoming darker as it reaches its final instar. It has a few scattered hairs on its surface, the normal arrangement of which is shown in the figure.

The entire head, excluding the mandibles is as broad as long; the *epicranial plates* (*ep l*) are large and are rounded laterally. The *epicranial suture* (*e s*) is very well marked, there being a gap between the epicranial plates at the base of the head; the lateral arms (*l es*) of this suture distinctly separate the plates from the frons. The *frons* (*fr*) is triangular in form and anteriorly there is a slight indication of an *epistoma* (*ep*)<sup>1</sup>. There is no indication of eyes or ocular pigment. The *antennae* (*a*) are present as stout papillae with two small tubercles at their bases. The *mandibles* (*mn*) are strongly chitinous, stout and triangular in outline. They are tridentate, the apical and subapical teeth being more acute in form than the smaller lower tooth, which is sometimes merely a prominence. There are four well-developed spines on each mandible arranged normally as figured. The *clypeus* (*cl*) is quite distinct and is longer laterally than in the median line; it is devoid of spines. The dome-shaped *labrum* (*c*) is well covered with spines especially at the apex, the arrangement as figured is normal and characteristic.

*Ventral view* (Fig. 8). The *maxillae* (*A*) are well developed and, with the exception of the galea and subgalea, all the sclerites are distinct. The *cardo* (*cd*) is stout and club-shaped, and unites with the larger stipes (*st*), the latter having several strong spines as indicated on the figure. The *palpifer* (*f*) is short, slightly broader than long and bears the *maxillary palpus* (*mx p*) which is represented as a stout elongate unsegmented lobe, fringed with papillae at its apex. The *lacinia* (*lc*), fused as it is with the galea (*gl*) and *subgalea* (*s gl*) is in the form of an elongate lobe, the interno-lateral face of which is fringed with lacinial teeth. The *labium* (*B*) is large and slightly broader than long, the broadest line being nearer the base. The *submentum* (*sm*) comprises most of the labium and is rounded laterally; there are a few strong spines on its surface as indicated. The *mentum* (*mt*) is triangular, the apex of which reaches beyond the middle of the head, this sclerite also has scattered

<sup>1</sup> See previous footnote.

spines on its surface. Anteriorly there is a faint suture indicating the division of the mentum and *prementum* (*pm*): the latter sclerite is very narrow and has a ridged free margin. The *labial palpi* (*lp*) are short stumpy unsegmented lobes with their apices fringed with papillae.

#### *Thorax.*

The three sclerites of the thorax are clearly defined, the prothorax being slightly reduced. The *prescutum* (*psc*), the *scutum* (*sc*) and the *scutellum* (*scl*) are only feebly indicated. The *pleurites* (*pl*) as a whole are well defined in the thorax of mature larvae but the individual constituents are not indicated.

There is a biforous spiracle at the junction between the prothorax and mesothorax; this will be described later.

#### *Abdomen.*

The abdomen possesses 10 distinct segments, the sutures becoming less distinct anally: in each of the segments of the notum the elements are indistinct. The 10th segment is considerably reduced and serves occasionally as an organ of locomotion. The *pleural groove* (*plg*) is well marked and there are indications of the *hypopleural fold* (*hlp*) and the *sternellar fold* (*st*). Hairs are scattered over both abdomen and thorax.

#### *Spiracles* (Fig. 9).

There are eight pairs of spiracles. The first pair situated between the prothorax and mesothorax are *biforous* in form (Fig. 9 A). Each consists of the annular sclerite or *peritreme* (*pr*) which surrounds it, the spiracular opening (*o*) which leads into the *atrium* (*a*): posteriorly this leads into a double chamber, the compartments being separated from each other by a slight longitudinal partition; transversely there exists a series of chitinous *trabeculae* (*tr*). A closing apparatus is present but this is best described in the abdominal spiracles. There is no spiracle present on the meso- or metathorax, neither is there any indication of this structure in the last three abdominal segments. Each of the other abdominal segments bears laterally and somewhat anteriorly a pair of spiracles of normal structure (*i.e.* not biforous). Each (Fig. 9 b) consists of a spiracular opening (*o*) which leads into the *atrium* (*a*) and this extends posteriorly into an oval chamber across which are arranged 6 or 7 transverse *trabeculae* (*tr*). At the inner end of the atrium is the closing apparatus consisting of a chitinous bow (*ch*) the base of which unites to form a chitinous band around the trachea. The longer chitinous

arm extends posteriorly, while the short one lies in an antero-lateral position. Occlusor muscles are attached to these rods and function in opening and closing the spiracular opening.

#### 6. THE PUPA (Fig. 10).

The pupa is soft, of creamy white colour, and is capable of active movement when touched or exposed to changes in temperature. It varies in length from 2.0–2.5 mm., and usually lies on its side within the pod. The head is bent ventrally and the elongate rostrum extends to the abdomen. As in the adult the size of the rostrum indicates the sex of the pupae. A few scattered bristles are visible in the anterior region, but the arrangement of these does not appear to be characteristic. The pupal integument is densely covered with minute papillae. The antennae (*a*) extend from the base of the rostrum in a latero-anterior direction. The legs are folded ventrally, the prothoracic (1) and mesothoracic (2) legs in a more or less anterior position, while the tarsi of the metathoracic (3) legs extend posteriorly to the 7th abdominal segment. The tips of the elytra extend to the 6th abdominal segment, the hind wings being completely concealed by the elytra. The abdomen has 10 distinct segments, the 10th segment being extremely rudimentary, appearing as a mere tubercle. The 9th abdominal segment terminates in two prominent caudal spines.

#### 7. LIFE-HISTORY.

##### *Hibernation.*

*Apion ulicis* hibernates as the adult and in this stage has been beaten from gorse bushes through the winter. It does not hibernate normally within the pod as stated by Bargagli(2). Examination of debris and soil beneath the bushes for hibernating weevils yielded negative results but close observation of the branches revealed adult specimens—their greyish colour resembling small buds—at the points where buds and spines leave the branches. During a spell of sunshine these adults become more active and are easily observed. Dissection of the reproductive organs of about 500 females periodically during the winter months showed the absence of sperms in the spermatheca and also revealed the immature condition of the ovaries, thus confirming field observations that mating had not taken place. Further, it was not until the end of February when the weevils became more active and were observed nibbling the branches and young shoots, that any appreciable quantity of food was observed in the alimentary canal.

*Mating.* On March 2nd sperms were first found in the spermatheca of a single female but, despite daily observation, mating was not observed in the field until March 26th. Later, in April and May it was commonly observed. Pairing was not witnessed after the end of May. Prior to mating, the male with rostrum held in a ventro-posterior position follows the female, eventually seizing it by placing the claw of one of its anterior legs on the anterior ridge of the prothorax. Continuing this action for some time the male eventually mounts and copulation takes place. Within a glass tube or cage the male and female pair at intervals, but under natural conditions, from the comparative ease with which the female can remove the male by pushing under the spines of the gorse plant, it would appear that a single pairing normally occurs. A virgin female, after a single copulation had taken place, was found to have its spermatheca filled with sperms. In many cases it was observed that the male, during copulation, scraped the scales from off the back of the female with its tarsal claws, thus resulting in a black fertile female. This accounted for the quantity of black females found in the field and all such females were found to be fertilised. It was, however, later noticed that the removal of scales did not occur during every copulation. The period from the date of mating to the time of oviposition varied from 30–42 days.

*Oviposition.* The gorse did not come into flower in Harpenden until mid-April, and pods of any appreciable size (the anthers and calyx of flower being still retained) were not observed until mid-May. Daily observations of the gorse for oviposition were continued throughout May and the first instance was observed on May 11th. According to Goureau<sup>(8)</sup> oviposition took place in February and March in S. France. As illustrated in Fig. 11 the female first bores a hole in the pod with its rostrum. A large series of counts taken indicates that no particular portion of the pod is chosen for oviposition while frequently the weevil bores through where the calyx still surrounds the pod. It was clearly observed, however, that the weevil prefers a young pod and oviposition ceases on bushes where the pods have become hard and black. The time taken for boring the hole varied between 1 and 5 hours, feeding naturally took place during this operation, for on removal of the rostrum the mandibles were observed still at work. After the withdrawal of the rostrum, the female turns around and orientates itself by means of its anal end until the ovipositor is placed within the hole (Fig. 12). Occasionally the ovipositor is placed within a hole recently made by another female, while on the other hand, several attempts at orientation were observed to be

entire failures. One particular instance of what might be termed "love's labour lost" was witnessed in which the female, after spending from 2-7 p.m. boring the hole, attempted without success, for half an hour to place its ovipositor within the hole in the pod and it finally walked off. Instances of this kind usually result in the eggs being deposited outside the pod but as will be seen later, eggs thus laid do not develop. There is no attempt whatever to close up the hole in the pod which can clearly be seen under the binocular microscope. As the pod develops these holes become closed and it is very difficult to find a trace of a hole in a mature green pod, while in a mature dark pod detection is impossible. The eggs are laid in batches within the pod (Fig. 13), the normal number per batch being 6-8 eggs. More than one batch of eggs frequently occurs within a single pod; these are probably instances of two females ovipositing in the same pod and even in the same hole. The number of eggs per pod obtained from counts taken from a large number of pods varied from 1-23. Oviposition continued at Harpenden during May until early August. Experiments arranged to ascertain the number of eggs laid by a single female were rendered void owing to the fact that all the eggs were not placed within the pods.

#### *Incubation.*

The incubation period was 26 days ( $\pm 4$ ) during which the egg changes from an elongate yellow form and assumes a spherical pearly white appearance. The form of the embryo within the egg can be seen through the delicate chorion about the 20th day. The embryo is curled back upon itself, the head and anal region practically touching each other. On hatching the chorion splits in the mid-dorsal region of the embryo, the latter pushing the chorion over both head and anal end as it emerges, and eventually tugging itself away from the remaining delicate chorion.

#### *Larval Period.*

The young larva, after emergence, wriggles its way to the base of the seed, where the soft funicle of the seed affords its first food. The larva shows definite negative phototropism and, because of this fact and also that frequent disturbance is detrimental to the development of the larva, investigations with a view to ascertaining the number and nature of the instars gave unsatisfactory results. Further, after the larvae had pierced a hole in the seed coat, in several instances it entered the seed and was thus lost to observation. It is, however, certain that the first moult takes place on the 9th or 10th day after emergence and the final

moult occurs just prior to pupation. At the last moult the comparatively large head capsule is discarded and remains close to the pupa. The entire larval period is 45 days ( $\pm 5$ ).

#### *Cocoon formation.*

Despite the fact that the larvae is enclosed within the gorse pod and sometimes within the seed coat, during its last instar it proceeds to make a cocoon. The cocoon consists of a brown glutinous material forming a distinct chamber closely surrounding the pupa. Mature larvae were observed making cocoons and it was seen that a light brown material exuded anally. Dissection proved that the material practically filled the alimentary canal and offered a marked contrast to the green contents of the alimentary canal of younger larvae. The substances exuded spasmodically indicating definite expulsion by the larva which removed the material from the anus by means of its mandibles. In the region of the mouth-parts the excreted mass evidently received a salivary secretion, for the entire surface of the mouth-parts was bathed in a colourless fluid which welled up at intervals and was mixed with this anal secretion. The mixture was then arranged into a cellular chamber with distinct walls and a roof which eventually encloses the larvae. The necessity for this cocoon appears to be obscure unless it assists in hindering the passage of parasitic Hymenopterous larvae which have been observed isolated from enclosed pupae. It may also be necessary to maintain a constant humidity.

#### *Pupal Stage.*

Continuous examination of pods from the Harpenden common throughout the summer, yielded the first pupa on July 8th. Pupae predominated in the pods during the latter part of July and in August, a few were found as late as October 9th. Under laboratory conditions the pupal stage was 10 days ( $\pm 2$ ). The pupae remain white until the last few days of the pupal period. Pigment first appears in the eyes and rostrum, later it develops in the thorax, the coxae, apices of the femora, in the tibia and the tarsi. On opening pods weevils with their elytra and abdomen still white have walked out.

#### *Adults.*

The number of adults per pod varies considerably and the results of numerous counts became so interesting that it was decided to examine in detail 500 infected pods taken at random on the Harpenden Common. The normal number of individuals per pod was 4.6, the number varying

from 1-16. The normal arrangement of the adult weevils within the pod prior to emergence is seen in Fig. 14. The weevils are usually laterally placed, and when numerous the method of packing is extraordinarily efficient. The partitions of the cocoons can also be seen in the photograph. The adult weevils emerge from the pod when the pod dehiscence on fine sunny days. The crackling of gorse pods in the sunshine is a familiar sound in all gorse areas. Normally the seeds are hurled into the air when the pods burst, so that in the case of infected pods, the weevils are similarly thrown out and immediately become active. Despite the possession of strong biting mandibles the adults are incapable of emerging from the pod by their own efforts. This fact was strongly suggested by the discovery in April and May of quantities of unopened pods containing dead adult weevils. The fact has also been fully proved by retaining quantities of unopened infected pods in the laboratory, and on examination after several months later no adults had emerged, but when the pod was opened mechanically the weevils immediately became active. Further observations in the field show that a certain number of pods do not open naturally for some reason or other, they are retained on the plant or fall to the ground. Such pods have been collected during the Winter and Spring, and some have yielded unattacked seeds; some, dead or moribund weevils and others, weevils which became very active as soon as the pod was opened. A sample of old unopened pods was examined in May 1927 and these yielded live adults obviously from the 1926 generation. The female reproductive organs of these weevils when examined were found to be quite immature, while normally the females were ovipositing in the field. In view of the fact that the gorse pod depends on bright sunny weather in order to dehisce, it is probable that as the result of a wet Summer and Autumn, large numbers of weevils will suffer the fate of being thus imprisoned. Weevils emerging normally in late Summer and Autumn can be found quite active on the gorse plant on sunny days, but at the first indication of frost they become sluggish and are difficult to see on the plant. On very warm days in Spring and Summer they will readily take to the wing. It may be of interest to note that they strongly exhibit positive phototropism.

#### 8. DAMAGE.

The actual damage the adult weevils do appears to be negligible, it is a mere browsing and puncturing of the spines and softer portions of the plant.

From the 500 collected pods previously mentioned the damage caused by the larvae to infected pods was ascertained. It was found that 69.4 per cent. of these pods had their entire contents devoured by the larva of *Apion ulicis*, while 18.6 per cent. had a single whole seed remaining, 10 per cent. had two entire seeds and 2 per cent. had three seeds still intact. It is especially interesting to note the economy of food by the larvae which occurs under certain conditions. The number of seeds in a normal pod varies from 4-7. From the data collected it was seen that 3 larvae could devour the entire contents of a normal pod, while in several instances as many as 16 larvae had developed and produced apparently quite normal adults. A few of these were slightly smaller in size but there was no marked difference.

It is obvious that to ascertain a normal percentage of pod infestation counts would have to be taken after oviposition had ceased. This was done on three occasions at Harpenden when 200 pods were collected at random and on examination it was found that 88, 77 and 82 per cent. of the pods were infected respectively.

#### 9. SURVEY OF THE DAMAGE IN GREAT BRITAIN.

It is clear that to secure absolute figures for the percentage pod infestation of *Apion ulicis*, for any given area or for Great Britain as a whole, would involve far more work than a single person could undertake.

It was, however, felt desirable to ascertain the percentage pod infection *possible* under conditions in Great Britain, and further to secure some indication of regional distribution.

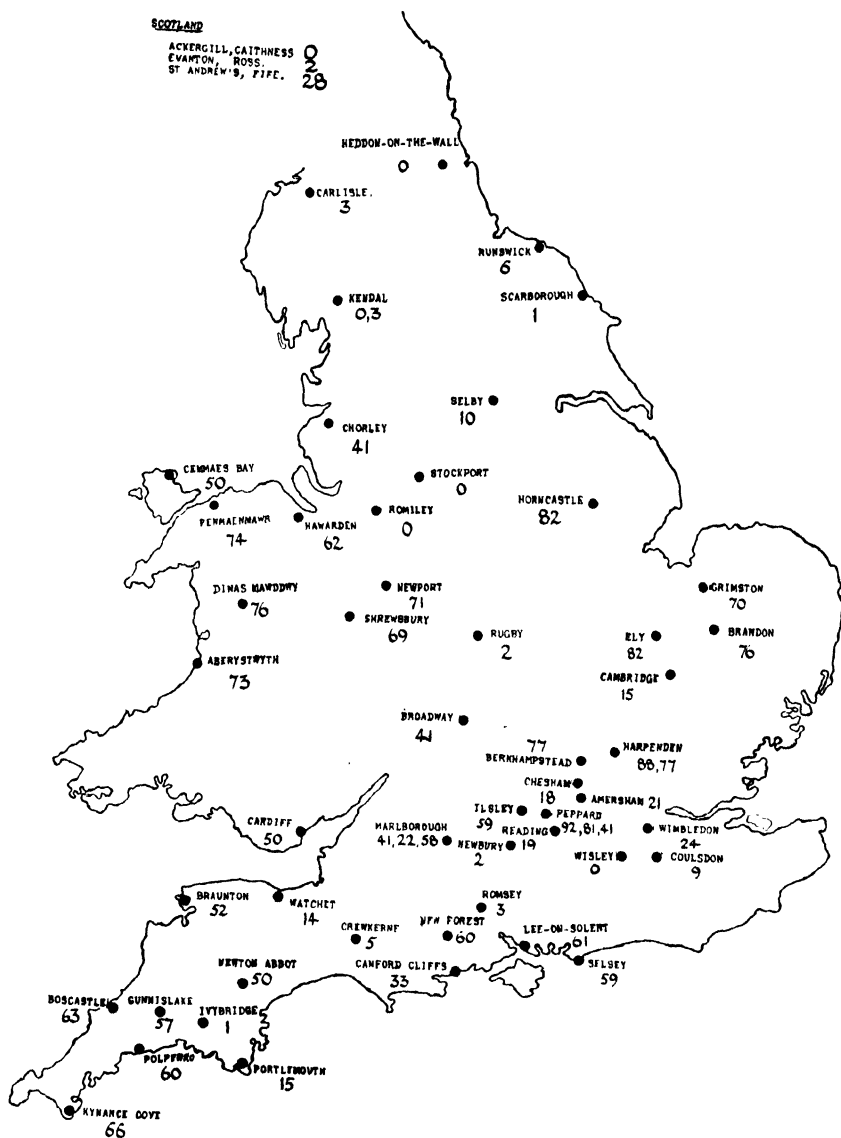
#### *Survey of infestation of Apion ulicis in Great Britain.*

No.	County	District	% pod infection	Remarks
1	Caithness:	nr Wick, Ackergill ...	0	Extreme N. Scotland. 30 % L
2	Ross:	Evanton ...	2	30 % L
3	Fife:	St Andrews...	28	—
4	Northumberland:	Heddon-on-the-Wall	0	32 % L
5	Cumberland:	Carlisle ...	3	—
6	Westmoreland:	Scout Scar, Kendal ...	3	6 % L
7	"	Paddy Lane, Kendal ...	0	—
8	Yorkshire:	Runswick nr Whitby ...	6	21 % L near seashore
9	"	Scarborough ...	1	7 % L
10	"	nr Selby, Riscal Common ...	10	—
11	Lancashire:	Chorley ...	41	32 % L
12	N.W. Derby:	Stockport ...	0	Alt. 800 ft. 16 % L
13	Anglesey:	Cemmaes Bay ...	50	—
14	Carnarvon:	Penmaenmawr ...	74	Alt. 700 ft.
15	Flintshire:	Hawarden ...	62	—
16	Cheshire:	Romiley ...	0	Alt. 450. 1 % L pods black with soot

No.	County	District	% pod infection	Remarks
17	Lincoln:	Horncastle ... ..	82	2 % L
18	Merioneth:	Dinas Mawddwy ... ..	76	—
19	Salop:	Newport ... ..	71	3 % L
20	"	S. E. Shrewsbury, Fetch Hill ... ..	69	25 % L
21	Warwick:	Rugby ... ..	2	15 % L
22	Norfolk:	Grimston ... ..	70	—
23	Suffolk:	Brandon Common ... ..	76	—
24	Cambridge:	1½ miles S. Ely ... ..	82	3 % L
25	"	Cambridge University Farm ... ..	15	Taken from gorse hedge, no other gorse for several miles
26	Cardigan:	Aberystwyth ... ..	73	—
27	Worcester:	Bayliss Hill, Broadway ... ..	41	5 % L
28	Hertford:	Harpenden ... ..	88	—
29	"	" ... ..	77	—
30	"	" ... ..	82	—
31	"	Berkhampstead ... ..	77	—
32	Buckingham:	Chesham ... ..	21	48 % L
33	"	Amersham ... ..	18	—
34	Berks:	Isley ... ..	59	10 % L
35	Essex:	Epping ... ..	85	6 % L
36	Bucks:	Ibstone ... ..	18	6 % L
37	Oxford:	Peppard Common ... ..	81	—
38	"	" " ... ..	92	Taken from old bushes, not known to have been burnt. 2 % L
39	"	" " ... ..	41	Adjacent to the foregoing, bushes periodically burnt
40	Berks:	Reading ... ..	19	—
41	"	Padworth ... ..	64	—
42	"	Newbury ... ..	2	12 % L
43	Wilts:	N.W. Marlborough ... ..	58	12 % L
44	"	S. Marlborough ... ..	42	—
45	"	S.W. Marlborough ... ..	22	13 % L
46	Surrey:	Wimbledon Common ... ..	24	21 % L
47	"	Wisley ... ..	0	55 % L
48	"	Esher Common, nr Coulsdon ... ..	9	68 % L. Some <i>Apion</i> half eaten
49	Somerset:	Doniford, nr Watchet ... ..	14	11 % L
50	"	Crewkerne ... ..	5	30 %
51	Hampshire:	Romsey ... ..	3	56 % L
52	"	New Forest ... ..	60	29 % L
53	"	Lee-on-Solent ... ..	61	—
54	Dorset:	Canford Cliffs ... ..	33	21 % L. Some <i>Apion</i> half eaten
55	Devon:	Braunton ... ..	52	19 % L
56	"	Newton Abbot ... ..	50	—
57	"	Ivybridge ... ..	1	36 % L
58	"	Portsmouth ... ..	15	27 % L
59	Cornwall:	Gunistlake ... ..	57	—
60	"	Boscastle ... ..	63	22 % L
61	"	Polperro ... ..	60	—
62	"	Kynance Cove ... ..	66	—

L=pods also attacked by lepidopterous larvae.

An organised survey has been made possible through the kind assistance of a number of persons to whom the writer is greatly indebted. Samples of 100 pods each gathered at random over gorse areas from



Map. Distribution of *Apion ulicis* in Great Britain.  
(Figures indicate percentage pod infection.)

54 selected districts through Great Britain, have been secured. From this number of pods there will be a probable error of  $\pm 10$  per cent. infestation. These pods have been examined for *Apion ulicis* by the writer and the results are indicated in the table and on the map. It is then seen that as high as 92 per cent. pod infestation has occurred, but it should be noted that this particular infestation in Oxfordshire was on old bushes which as far as could be ascertained were not known to have been burnt, at least for very many years. Adjacent to these bushes was another area of gorse which had periodically been burnt and here the percentage was reduced to 41. The habit of burning gorse in Great Britain, then, undoubtedly decreases the efficiency of *Apion ulicis* in destroying gorse seeds, and no doubt accounts for many of the low percentages recorded. Further, the presence of Lepidopterous larvae reduced the percentage of attack by *Apion ulicis*, for in many cases the caterpillars had devoured the entire contents of the pod, larvae or pupae of the weevil included. Several cases of half eaten pupae were noticed. It is interesting to note that from the samples received no really high infestation of *Apion ulicis* was recorded from the North of England and Scotland. This result needs confirmation.

The infestation on Harpenden Common has been under observation by Dr Imms for some years, and it is stated that in certain years it has been practically impossible to secure a sample of sound seeds. A few counts were made in July 1926 by H. T. Pagden, and though the numbers counted were small in comparison with the present year, the pod infestations taken from 7 different counts averaged 77 per cent.

#### 10. TESTS ON ECONOMIC PLANTS.

Before an insect can be introduced into a new country it is, of course, essential that the particular insect should undergo most critical tests on all the plants of economic importance that there is the slightest possibility of it attacking. This aspect of the work has therefore received primary attention. The method adopted for all work of this kind is to subject the insect concerned to a "starvation test" when death of the insect on the particular economic plant concerned is the only criterion that will justify further consideration of that species. It is felt that the selective faculty of the insect cannot be relied upon in this matter, for one cannot assume that if an insect is specific in its host plant in the field, it will remain so under all conditions. Not only are "starvation tests" carried out in this country, but all insects successfully standing

these tests in this country will be submitted to similar tests in their new environment abroad.

The technique used to test *Apion ulicis* can be grouped into three sections.

I. *To test if eggs of Apion ulicis laid outside the pods could develop.*

As previously mentioned it was found that under unfavourable conditions *Apion ulicis* laid its eggs outside the pod, on the branches and elsewhere. It was very important to ascertain if these could develop. Eggs thus laid were collected and arranged on the outside of the pods in the field, the eggs being protected with muslin bags. Three series of 50 eggs each were then tested in turn. On each occasion in two or three days the eggs had shrivelled to almost unrecognisable masses. A similar series of experiments was carried out with eggs normally laid within the pod and carefully removed and placed on the outside of the pod in the field. These all suffered a similar fate to the preceding. It was next desirable to test if newly hatched larvae could penetrate the pods from the outside. All newly hatched larvae placed on the outside of pods perished within a day or so.

From these results it was obvious that the gorse pod played an essential part in the life-history of the weevil, for without it development was impossible. Thus it followed that the only economic plants to be considered in the tests were pod-forming species of the Leguminosae.

II. *To test whether Apion ulicis would oviposit in pods of other leguminous plants.*

The pod-forming plants which have been considered are: Broom (*Cytisus (Sarothamnus) scoparius* Link.), Lupin (*Lupinus*), Broad Bean (*Vicia faba*), Kidney Bean (*Phaseolus vulgaris*), Garden Pea (*Pisum sativum* L.), Lucerne (*Medicago sativa*) and Wild White Clover (*Trifolium repens* L.). It was found that the factor of captivity could be ignored in the case of *Apion ulicis*, for females readily oviposited in a gorse pod within a test tube. It was noted, however, that under similar conditions *Apion ulicis* would not oviposit in pods of other plants. Three cages of growing plants of each of the above species were arranged and 30 ovipositing females together with 10 males were put in each cage. A cage of *Ulex* was kept as control. Examination of pods after 2 months interval resulted in practically every gorse pod being infected whereas no sign of oviposition in the pods of the other plants was witnessed. At this

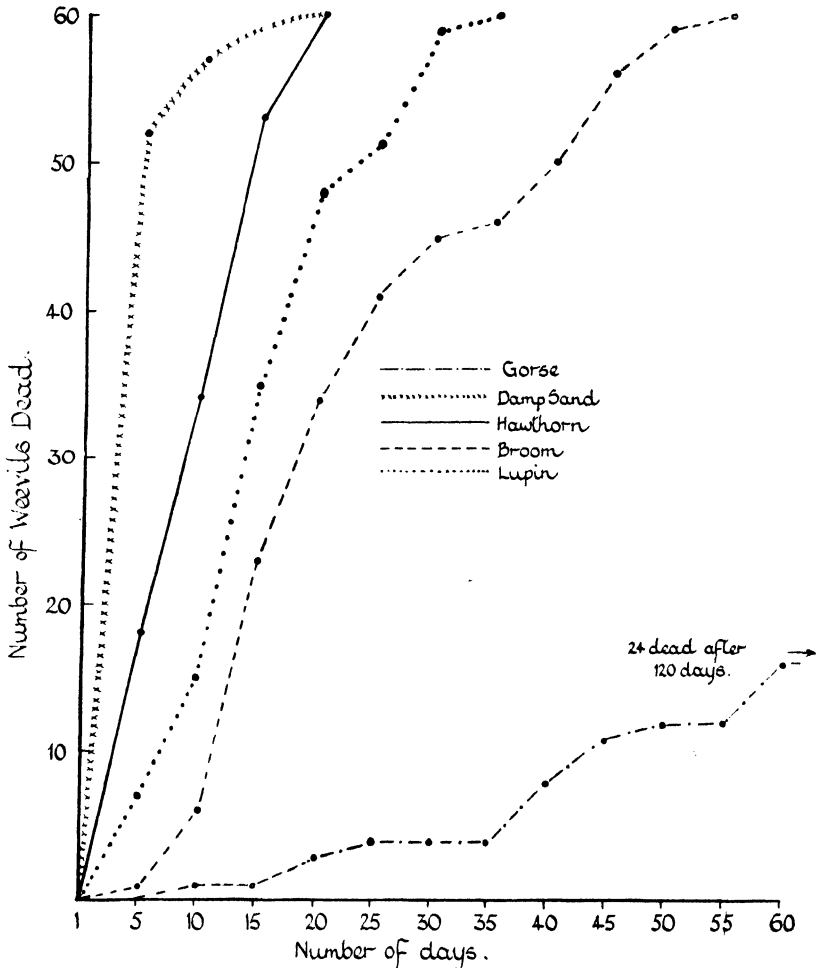
date no live adults could be found on the "tested" plants, while 28 females and 5 males were still alive on the gorse. This test was further elaborated in that both eggs and larvae of different ages were placed within the pods of tested plants. Pods of Broom, Lupin, Broad Beans, and Peas only were found practicable for the tests. A series varying from 10-20 pods of each plant was experimented with; eggs, and larvae of varying sizes, were put in each pod. Examination of pods later showed that while in some instances slight nibbling of the seeds of the tested plant had taken place, not a single larvae developed to the pupation stage.

### III. *To test if adult weevils can survive on other leguminous plants.*

To secure comparable results the method shown in the photograph (Fig. 15) was adopted<sup>1</sup>. Sprigs of the plants concerned were cut and enclosed within a lamp-glass, the top of which was covered with a muslin cap; the stalks projected into a test-tube of water. Each plant was tested in triplicate, 20 weevils being placed in each. Counts were taken every 5 days, and the results have been plotted in Graphs I and II. In Graph I the weevils used were those of the 1926 generation and thus the tendency of the weevils to die off on the gorse is observed. There is, however, a striking difference between the death rate on gorse and that on other host plants. The host plants tested were confined to lupin and broom, because at the time when this experiment was commenced there was not a sufficient supply of the other plants available. Graph II gives the results of a larger series of experiments, where the weevils used were those secured from the pods of gorse before they had fed on their natural host plant. It was felt that these tests would give more reliable results. It is regretted that after the 45th day an accident to the tray of experiments rendered further procedure impossible. It is fortunate, however, that the experiment was sufficiently far advanced to give significant results and the final termination of the curves can be approximately assumed. Repetition was impossible owing to the fact that frost soon occurred and the weevils commenced hibernation. Actual nibbling of the plants of lupin and broom was observed, but it is evident from the curves that this was not beneficial to the weevils. In fact from the position of the "damp sand" and "hawthorn" curve there is a strong indication that some of these host plants may actually be detrimental to the welfare of the weevil. It is quite obvious that under the conditions

<sup>1</sup> The technique is that used by C. T. Gimmingham in research on insecticides and a detailed description of the same will be shortly published.

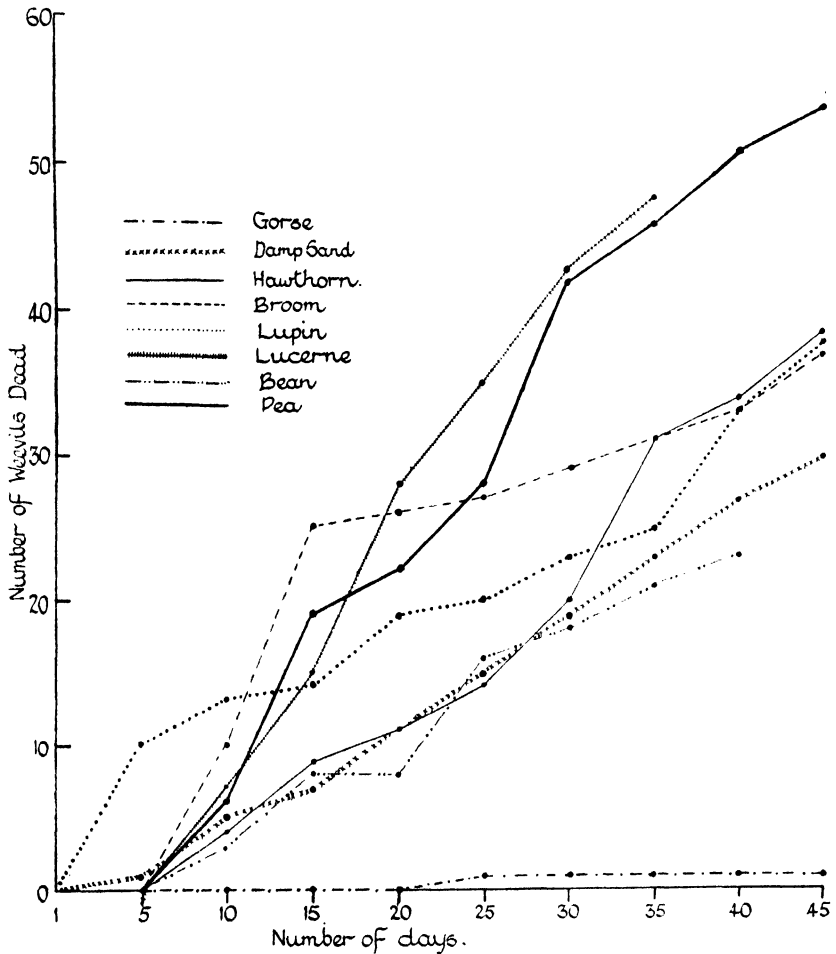
of these tests these plants cannot serve as food for *Apion ulicis*. It is interesting to note that *Apion ulicis* has been recorded from *Ulex nanus*, and Bargagli(2) states that it has been observed on *Genista tinctoria*.



Graph I. Death-rate of *Apion ulicis* (old specimens) on economic plants.

Regarding the occurrence of *Apion ulicis* on broom (*Cytisus scoparius*) it should be stated that on Harpenden Common there is a small area of broom among the gorse. Periodical examination and beating of these bushes with a view to ascertaining the presence or otherwise of the

weevil, have yielded entirely negative results; despite the fact that *Apion ulicis* is in abundance on the adjacent gorse bushes.



Graph II. Death-rate of *Apion ulicis* (newly emerged) on economic plants.

## 11. PARASITES.

One of the most important points in biological control is the separation of a beneficial insect from its parasite, or hyper-parasite as the case may be. Thus the greatest possible care has to be taken lest the parasite be introduced into the new environment along with its host. Such a step might result in entire failure of the attempt. It is, of course,

also essential that parasites of the insect about to be introduced should not already exist in the new environment.

Observations have been made regarding parasites of *Apion ulicis* and a few have occurred in practically every locality. An estimation of the percentage infestation has been made at Harpenden; the 500 pods previously referred to afforded the following data: 9 per cent. of the infected pods were infected with parasites, the number of parasites per pod varying from 1-8. The actual percentage of *Apion ulicis* parasitised was only 4. The degree of parasitism at Harpenden was quite the normal of other districts investigated.

The parasites proved to be all of one species<sup>1</sup>. This species was originally described by Goureau(8) as *Semiotus apionis*; the genus *Semiotus* Wlk., it may be added, has now become a synonym of *Semiotellus* Wstw. Dr Waterston, who kindly identified these parasites, however, states that while the parasites obtained from *Apion ulicis* agree perfectly with Goureau's description of *Semiotus apionis*, they do not belong to the genus *Semiotellus*, so that the generic position of this species will need to be ascertained.

There are three other records of parasites of *Apion ulicis*: *Pteromalus pirus* Wlk. and *Eulophus ulicis* Perr. both recorded by De Gaulle(3), and *Semiotus brevipennis* Walk. bred by Goureau (Dours. Cat. 102). All these records are from France.

## 12. SUMMARY.

1. The present study of *Apion ulicis* Först. is in reference to the use of this weevil in the control of *Ulex europaeus* in New Zealand: its synonymy and geographical distribution are dealt with.

2. The external morphology of the egg, larva, pupa and imago of *Apion ulicis* have been studied, special attention being devoted to the mouth parts of both adult and larva. The male and female reproductive organs are also described and figured.

3. The details of its life-history and feeding habits are given and an account of the damage caused by both adult and larva is included.

4. A survey of 62 districts in Great Britain has been organised and as high as 92 per cent. pod infection has been observed.

5. Primary attention has been given to the possibility of *Apion ulicis* attacking economic plants. It was found that only leguminous plants need be considered, and of these oviposition only occurred in

<sup>1</sup> Dr Waterston has since identified the species as *Splinterus leguminum*, Ratz.

podis of *Ulex europaeus*. Tests to ascertain the ability of *Apion ulicis* to thrive on other leguminous plants gave entirely negative results.

6. A parasite identified as *Splintherus leguminum*, Ratz. has been bred, a 4 per cent. parasitism was estimated. Three other records of parasites of *Apion ulicis* are quoted.

### 13. LITERATURE.

- (1) ALEXANDER, W. B. (1925). Natural Enemies of Prickly Pear and their Introduction into Australia. *Inst. Sci. and Indust. Melbourne, Bull.* No. 29.
- (2) BARGAGLI, P. (1884). Rassegna biologica di Rincofori Europei. *Bull. Soc. Ent. Ital.* xvi, 246.
- (3) DE GAULLE (1908). *Catalogue de Hymenoptères de France*, pp. 95, 102, 109.
- (4) ELLIOTT, E. A. and MORLEY, C. (1911). Hymenopterous Parasites of Coleoptera. *Trans. Ent. Soc.* p. 477.
- (5) FABRICIUS (1798). *Ent. Syst. Suppl.* p. 163.
- (6) FÖRSTER (1771). *Nov. Spec. Ins.* p. 31.
- (7) GOUREAU (1845). *Ann. Soc. Ent. France*, II, t. III, *Bull.* p. 79.
- (8) ——— (1847). Note pour servir à l'histoire des insectes qui vivent dans les gousses du genêt épineux (*Ulex europaeus*). *Ann. Soc. Ent. France*, II, t. V, p. 250.
- (9) GRADLER (1882). In Katter. *Ent. Mon.* p. 331.
- (10) GYLLENHAL (1833). In *Schönkerr Gen. Spec. Curc.* I, 269.
- (11) HOPKINS, A. D. (1911). 1. Contributions toward a monograph of the bark-weevils of the Genus *Pissodes*. *U.S. Dept. Agric. Bureau Ent. Technical Series*, No. 20, pt 1.
- (12) IMMS, A. D. (1926). The Biological Control of Insect Pests and Injurious Plants in the Hawaiian Islands. *Ann. App. Biol.* XIII, No. 3, 402-423.
- (13) KIRBY (1808). *Trans. Linn. Soc. Lond.* IX, 18.
- (14) KOEBELE, A. (1924). Report on Enemies of *Lantana camara* in Mexico and their Introduction into the Hawaiian Islands. *Ent. Bull.* xvi, H.S.P.A. 54-71.
- (15) PERKINS, R. C. L. and SWEZEY, O. H. (1924). The introduction into Hawaii of Insects attacking *Lantana*. *Ibid.* 1-53.
- (16) PERRIS, E. (1876). Nouvelles promenades entomologiques. *Ann. Ent. Soc. France*, v, t. VI, p. 175.
- (17) SCHILSKY and KÜSTER (1906). *Kaf. Europ.* Heft 43, p. 27.
- (18) THOMSON, G. M. (1922). *The Naturalisation of Animals and Plants in New Zealand*.
- (19) TILLYARD, R. J. (1926). The Progress of Economic Entomology with special reference to Australia and New Zealand. *Journ. Roy. Soc. Arts*, Nov. 12th, LXXV, No. 3860, 3-20.
- (20) WAGNER (1912). *Genera Insectorum*, 130.
- (21) Government of New Zealand (1900). *Noxious Weeds Act*.

## 14. EXPLANATION OF PLATES XV—XVII

## PLATE XV.

- Fig. 1. *Apion ulicis* Först. (female).  $\times 20$ .  
 Fig. 2 a. *Apion ulicis*: mouth parts of adult (dorsal view).  $\times 300$ . Lettering as Fig. 3.  
 Fig. 2 b. *Apion ulicis*: mandible of adult.  $\times 300$ . *ad m*, adductor muscle; *ab m*, abductor muscle; *c*, condyle; *gm*, ginglymus; *ph b*, pharyngeal bracon.  
 Fig. 3. *Apion ulicis*: ventral aspect of mouth parts of adult.  $\times 300$ . *sm*, submentum; *mt*, mentum; *mx p*, maxillary palp; *l p*, labial palp; *lg*, ligula; *lc*, lacinia; *p*, palpifer; *cd*, cardo; *sg*, subgalea; *st*, stipes; *mx*, maxilla; *mn*, mandibles.  
 Fig. 4. *Apion ulicis*: male reproductive organs (dorsal view).  $\times 36$ . *a*, aedeagus; *ag*, accessory gland; *e d*, ejaculatory duct; *t*, testes; *tr*, transfer apparatus; *r*, chitinous rod; *v d*, vasa deferentia; *m*, longitudinal muscles.  
 Fig. 5. *Apion ulicis*: female reproductive organs (dorsal view).  $\times 36$ . *ag*, accessory gland; *b c*, bursa copulatrix; *m*, vaginal muscles; *o*, ovariole; *od*, oviduct; *sp*, spermatheca; *sp d*, spermathecal duct; *vit*, vitellarium; *vg*, vagina; *t*, terminal filament; *u*, uterus; *r*, chitinous rod.

## PLATE XVI.

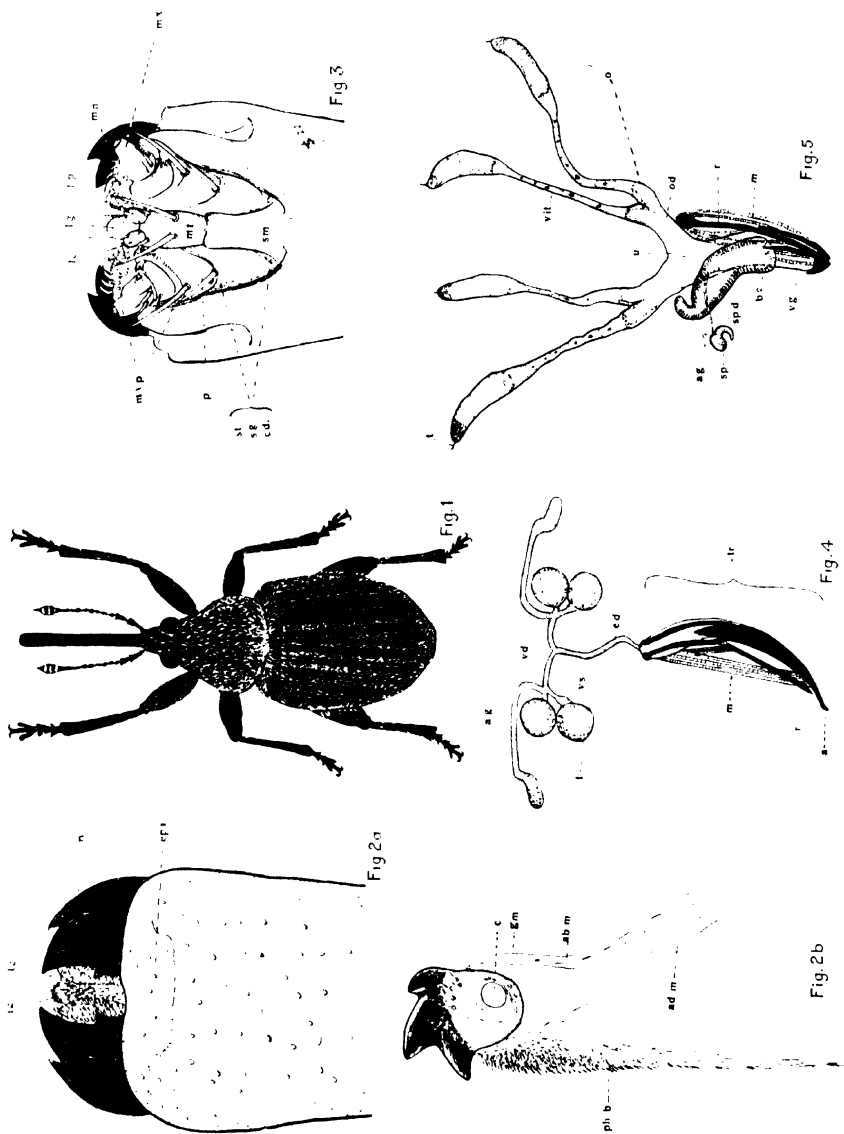
- Fig. 6. *Apion ulicis*: mature larva (lateral view).  $\times 36$ . *I*, prothoracic segt.; *II*, mesothoracic segt.; *III*, metathoracic segt.; *h*, head capsule; *p sc*, prescutal lobe; *sc*, scutal lobe; *scl*, scutellar lobe; *abd I*, 1st abdominal segt.; *abd X*, 10th abdominal segt.; *sp*, spiracle; *pl g*, pleural groove; *hlp*, hypopleural fold; *ep*, epipleural lobe; *st*, sternellar fold; *pl*, pleurites.  
 Fig. 7. *Apion ulicis*: mouth parts of larva.  $\times 135$ . *a*, antenna; *cl*, clypeus; *e*, labrum; *ep*, epistoma; *ep l*, epicranial plate; *e s*, epicranial suture; *fr*, frons; *mn*, mandible.  
 Fig. 8. *Apion ulicis*: mouth parts of larva (ventral view).  $\times 135$ . *A*, maxilla; *cd*, cardo; *st*, stipes; *sgl*, subgalea; *gl*, galea; *lc*, lacinia; *f*, palpifer; *mx p*, maxillary palpus. *B*, *mt*, mentum; *l p*, labial palp; *pm*, prementum; *sm*, submentum.  
 Fig. 9. *Apion ulicis*: spiracles of larva.  $\times 325$ . *A*, Biforous spiracle of pro- and meta-thorax; *B*, Abdominal spiracle; *a*, atrium; *ch*, chitinous bow; *o*, spiracular opening; *pr*, peritreme; *t*, tracheae; *tr*, trabeculae.  
 Fig. 10. *Apion ulicis*: pupa (female) ventral view.  $\times 36$ . *a*, antenna; *1*, prothoracic legs; *2*, mesothoracic legs; *3*, metathoracic legs; *abd IX*, 9th abdominal segt.; *c s*, caudal spine; *el*, elytra.

## PLATE XVII.

- Fig. 11. *Apion ulicis* (female) boring hole in gorse pod prior to oviposition.  
 Fig. 12. *Apion ulicis*: female with ovipositor in gorse pod.  
 Fig. 13. *Apion ulicis*: batches of eggs *in situ* within gorse pod.  
 Fig. 14. *Apion ulicis*: adults within gorse pods just prior to emergence; remains of cocoons visible.  
 Fig. 15. Portion of insectary with tray of "Starvation test" experiments *in situ*.

Figs. 11 to 15 are from photographs taken by V. Stansfield.

(Received December 8th, 1927.)









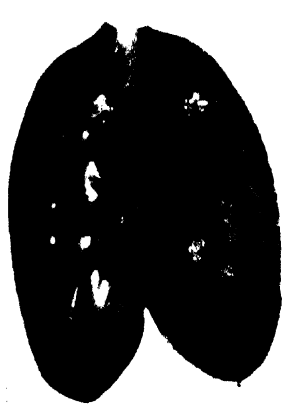
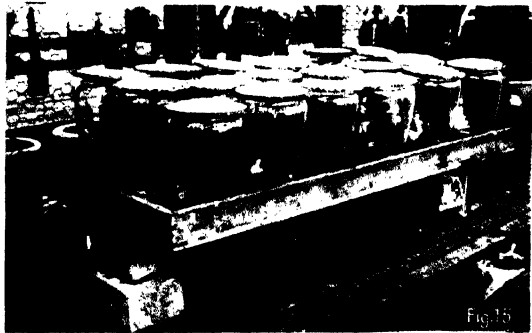
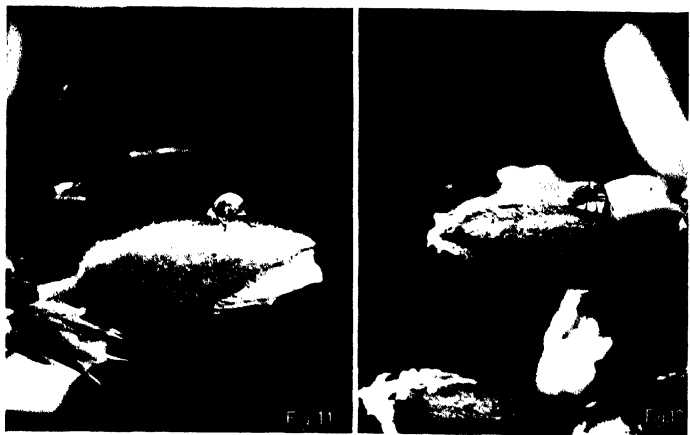


Fig.13



Fig.14



# THE DECOMPOSITION OF NAPHTHALENE IN THE SOIL AND THE EFFECT UPON ITS INSECTICIDAL ACTION

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(With 4 Diagrams and 1 Text-figure.)

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## INTRODUCTION.

NAPHTHALENE, either alone or in conjunction with other materials, has a certain reputation as a soil insecticide. It has been recommended for use against wireworms and leather-jackets; many experiments, however, have shown that its toxic action is uncertain under field conditions. Data to be presented indicate that in pot experiments in which thorough mixing of powdered naphthalene with finely divided soil is carried out by hand, naphthalene at moderate

concentrations is toxic to wireworms. Failure on a large scale may therefore be due to one or all of the following causes: (1) imperfect incorporation with the soil; (2) rapid disappearance from the soil either by decomposition or volatilisation; (3) a repellent action causing migration of the insects to positions where the vapour of the chemical is no longer effective. Naphthalene is known to be negatively chemotropic to many insects.

It has been shown<sup>(8)</sup> by experiments in sealed flasks that an atmosphere saturated with naphthalene vapour is not toxic to wireworms in a thousand minutes, its action being limited by the low vapour pressure of the compound. Thus naphthalene has three draw-backs for use in the soil as an insecticide: (a) it is slow in toxic action; (b) its vapour diffuses only slowly through the soil, in consequence of which its zone of toxic action is limited to a small volume; and (c) insects on coming into its zone of action are repelled and may escape from its toxic effects, unless the chemical is incorporated thoroughly with the soil to a fairly good depth.

It was early noticed in pot experiments that naphthalene disappeared from a good garden soil at a rate too rapid to be entirely accounted for by volatilisation. It appeared probable that decomposition by the action of micro-organisms in the soil was taking place. If this were so, the rate of loss would be expected to vary with the type of soil, and in certain soils the naphthalene might be decomposed too rapidly to be effective as an insecticide. Further, if the disappearance were due to bacterial action, the increasing numbers of naphthalene-decomposing organisms, which would result from repeated treatments of the soil by the chemical, would tend to make them progressively less effective against insects.

The experiments described here were designed to test these suggestions, and to attempt to determine the rate of decomposition of naphthalene in the soil. As a necessary preliminary, an investigation of methods for the determination of naphthalene in soil was undertaken.

The experimental work divides itself conveniently into two sections. The first part deals with the toxicity of naphthalene to insects in the soil, using wireworms as test subjects; and the second deals with the determination of naphthalene in soil and the investigation of its rate of decomposition.

The experiments were carried out in 1920 and 1921. Time was not available for their completion, but a number of points of interest emerged which it seemed advisable to put on record.

## EXPERIMENTAL.

*Pot and plot experiments on the toxicity of naphthalene to wireworms.*

*Method.* Lots of 2000 gm. of soil which had been passed through a 3 mm. mesh sieve were thoroughly mixed with powdered naphthalene in quantities varying from 0.1 to 0.0125 per cent. and put into glazed pots of an internal diameter  $5\frac{1}{2}$  in. and internal depth of  $6\frac{1}{2}$  in. A number of wireworms (8-10) were placed on the surface and allowed to penetrate or in some cases were put in first at the bottom of the pot and covered by the soil. The pots were placed in a cellar which varied little in temperature from day to day and were covered with brown paper which was occasionally moistened to prevent undue loss of moisture from the soil. The level of the soil was usually  $1\frac{1}{2}$  to 2 in. from the edge of the pot and the wireworms were unable to escape. The pots were examined at the end of one week.

At concentrations of 0.1 and 0.05 per cent. naphthalene was generally completely toxic and concentrations of 0.025 and 0.0125 per cent. were often lethal. The insects, however, varied in resistance to some extent according to the period of the year, and it is probable that just before moulting resistance is considerable, whereas immediately afterwards the wireworms appear more vulnerable to the action of the poison. As far as possible the insects chosen for experiment were of about the same size and in a state of activity; very light coloured individuals were rejected. In reading the data, it should be remembered that they refer to experiments carried out under conditions in which the insects had only a restricted area to move about in, and that they could not escape from the action of the poison, it is probable that in the open the results would have been somewhat less decisive.

The first set of experiments were designed to ascertain for how long a period naphthalene would retain its lethal properties in the soil. Different varieties of soil were chosen and, after putting through a 3 mm. sieve, treated with naphthalene in the usual way and examined from week to week till the toxic properties of the material were lost. A fresh batch of wireworms was added each week. The moisture content of the soil was determined from time to time and a little water added when necessary in an attempt to keep the moisture as constant as possible throughout the experiments.

The soils used were: (1) samples taken at different depths and a general sample to a depth of 9 in. from the manured and unmanured plots of Broadbalk field; (2) sample from Little Hoos field; (3) a richly



manured soil from a cucumberhouse; and (4) soil from an allotment in the laboratory grounds (a typical garden soil). The results are stated in Table I.

The data presented in Table I bring out several important points. It is clear, in the first place, that the effectiveness of the naphthalene depends on the type of soil; in the naphthalened "cucumber" soil all the wireworms were not killed at any of the concentrations tested (0.05–0.0125 per cent.), whereas with most of the other soils the higher concentrations were effective. Secondly, in well-manured soils, the naphthalene loses its toxic action more rapidly than in unmanured soils; thus, the naphthalened soil from Broadbalk manured plots (farm-yard manure) only retains its toxicity for about a week or ten days, whereas in soil from the unmanured plot toxicity persists for three weeks. Thirdly, treated soils from different depths from the manured and unmanured plots of Broadbalk retain their toxicity for different lengths of time; the top inch of soil from the manured plot destroys the toxic action of naphthalene in a little over a week, the soil from the 2nd to the 5th inch in a fortnight, and from the 3rd to the 7th inch in three weeks, whereas in the soil from 7–9 in. deep—just below the depth of ploughing—the toxic action persists into the 5th week. Similar results were obtained with the soil from different depths of the unmanured plot, except that in this case the toxic properties of the naphthalened soils from the top to the 7th inch persist for approximately three weeks and in the soil from the 7th to the 9th inch until the 5th week.

The natural deduction to be drawn from these data appears to be that the loss of toxicity is due to bacterial action and this is confirmed by experiments with sand and with sterilised soil. A garden soil (known as "allotment" soil) was mixed with sand (15 per cent.) and autoclaved for four periods of three hours each; lots of 2000 gm. were then naphthalened under as sterile conditions as possible and placed in pots of the usual dimensions; wireworms were introduced and each pot covered with two sheets of brown paper. A similar experiment in which clean sand was used and a control with naphthalened unsterilised "allotment" soil with 15 per cent. of sand were set up concurrently. It was found impossible to keep soil sterile under these conditions for more than a short period, as wireworms themselves introduced a contamination factor; nevertheless, the unsterilised soil retained its toxicity for only a week, whereas in the case of the sterile soil treated with naphthalene toxicity persisted for from two to three weeks and in the sand for a full four weeks.

These experiments were carried out under conditions where finely divided naphthalene was thoroughly incorporated with the whole bulk of soil, earlier experiments having indicated that when naphthalene was mixed with the top layer of soil only, the wireworms generally migrated to the bottom of the pot, out of range of the toxic action of the naphthalene, and so largely escaped injury.

Another set of experiments was set up to test whether the degree of fineness of the naphthalene materially affected its toxicity or persistence in the soil. A dry soil containing 10 per cent. of moisture was chosen in which 0.05 per cent. of finely ground naphthalene was known to persist for two weeks, and it was found that when crystals of naphthalene of the size of a pea were thoroughly incorporated, toxicity persisted for three weeks, but that when the particles were less in size than this the toxic effects disappeared as rapidly as with the most finely ground material. Here it is probable that the toxic effects and their persistence were accentuated by the dryness of this soil, the moisture content falling as low as 8.6 per cent. in the course of three weeks. The data given in Table II afford evidence of this. In each case "allotment" soil with 15 per cent. of sand was used, and apart from the variation in the moisture content the soil samples were similar.

Table II.

*Effect of moisture content of soil upon persistence of toxic action of naphthalene on wireworms.*

Moisture %	Naphthalene %	Percentage killed				
		First week	Second week	Third week	Fourth week	Fifth week
18.1	0.05	100	0	—	—	—
"	0.0375	90	0	—	—	—
"	0.025	90	0	—	—	—
17.8	0.05	100	0	—	—	—
"	0.025	100	0	—	—	—
15.8	0.05	100	0	—	—	—
10.8-8.6	0.05	100	100	80	23	0
"	0.0375	95	100	0	—	—
"	0.025	100	100	0	—	—
"	0.05	100	70	33	0	—
"	0.05	100	100	100	0	0

In considering the data in Table II, it should be realised that the resistance of the insects is undoubtedly affected by the dry conditions of the soil when the moisture content is 10 per cent. or below; nevertheless, these experiments afford clear evidence that the toxic action of naphthalene persists for a longer period under dry than under more

humid conditions of the soil, for the characteristic odour of the chemical disappeared from the moister soils in a period of about seven days, whereas in the drier soils it continued for some weeks. It is thus apparent that aridity imposes a limit upon the activity of the factor making for decomposition. It would be interesting to ascertain whether there be an upper limit to the range of water content of the soil above which naphthalene would be found to be relatively stable. This would possibly be the case, as free access of oxygen, which water-logging would prevent, would appear to be requisite for decomposition; it is, however, questionable whether the retardation would take place much below the water-saturation point and as the latter varies with the type of soil, the mere expression of the moisture values without reference to the soil type would afford no indication of the stability or otherwise of naphthalene in any particular soil.

By permission of Mr J. C. F. Fryer, an experiment on a larger scale was carried out in the grounds of the Plant Pathological Laboratory of the Ministry of Agriculture. Three plots of 1 square yard were divided off by corrugated sheeting sunk to a depth of 1 ft.; the soil to 12 in. deep in two plots was thoroughly mixed with naphthalene equivalent to 0.056 and 0.028 per cent. on the soil respectively, the third plot being left as a control. About 200 wireworms were placed in each plot and after a period of nine days the soil was gone through and the wireworms recounted. The central plot was subsequently treated with 0.019 per cent. of naphthalene.

I am greatly indebted to Mr E. H. Hodson, now of the Seale Hayne Agricultural College, for supervising these experiments and undertaking the laborious task of making the wireworm counts. The results are given in Table III.

Table III.

*Small plot experiments on toxicity of naphthalene to wireworms.*

(200 wireworms added to each plot.)

Soil treated to 1 ft. deep. Examination after 10 days.

Amount of naphthalene added	No. of wireworms recovered from each plot	No. unaffected	No. moribund
Control (untreated)	150	150	—
0.056 % (15 cwt/acre)	154	2	152
0.028 % (7.5 cwt/acre)	150	5	145
*0.019 % (5.0 cwt/acre)	167	86	81

\* The wireworms in this plot found unaffected were put back, and the plot re-examined after a further seven days; 78 were found alive and 8 dead; after putting back the "unaffected" for another month no further deaths were noted.

*Experiments on re-additions of naphthalene to soil.*

If the disappearance of naphthalene from soil be due to bacterial action, the enhanced number of naphthalene-decomposing organisms resulting from the treatment should lead to a more rapid decomposition of doses subsequent to the first, with a consequent lowering of toxicity. Four small experiments were set up to test this view. Two lots of 500 gm. each of "allotment" soil with an addition of 15 per cent. of sand were treated with 0.05 per cent. and two with 0.025 per cent. of naphthalene and put in glass jars of about 600 c.c. capacity fitted with screw caps. Wireworms were introduced into each. After examination at the end of the first week the soils were re-treated with the same amounts respectively, a fresh supply of wireworms being introduced each week. The naphthalene from the two higher concentrations disappeared too slowly to give conclusive results, but the two experiments with the lower concentrations indicate that the second dose of naphthalene was less effective than the first. The data were as follows:

Naphthalene added 1st week %	Deaths 1st week %	Naphthalene added 2nd week %	Deaths 2nd week %
0.025	70	0.025	0
0.025	100	0.025	25

*Loss of toxicity of naphthalene not due to volatilisation.*

Experiments were set up in which naphthalened "allotment" soil and sand containing 15.4 per cent. moisture, was kept in glass jars closed with screw caps. The soil was examined each week and a fresh supply of wireworms introduced. After the first addition no further naphthalene was added. The results obtained are given in Table IV.

Table IV.

*Duration of toxicity of naphthalene in closed vessels.*

Percentage naphthalene in soil	Percentage deaths		
	First week	Second week	Third week
0.05	100	100	0
0.05	100	100	0
0.025	100	0	—
0.025	100	0	—
Controls	0	0	0

Reference to Tables I and IV shows that in the experiments carried out in open pots with a similar soil of about the same water content, the toxicity of naphthalene at concentrations of 0.05 and 0.025 per cent. does not persist beyond the first week, although in the closed vessels, naphthalene at concentrations of 0.025 per cent. disappears in this time, the higher concentration (0.05 per cent.) persists for a further week. The more rapid disappearance of the higher concentration in open pots would not appear to be due to volatilisation as the vapour pressure of naphthalene is very low, but it does point to the fact that either free access of oxygen is essential for the decomposition of the chemical, or that the factor making for decomposition is not so active in closed as in open vessels. The results obtained in closed vessels indicate that the loss of naphthalene from the soil is mainly due to some factor inherent in the soil rather than to volatilisation.

*Experiments on the stabilisation of naphthalene in soil.*

For the purpose of controlling pests or disease organisms in the soil by chemical treatment, it is essential that the chemical should persist in the soil sufficiently long for its toxic action to be complete; but not for so long a time as to be detrimental to the crop following the treatment.

Two methods suggest themselves as suitable for stabilising naphthalene:

(1) The incorporation with the naphthalene of some other chemical or antiseptic.

Although not fully explored this method has so far not given very successful results.

(2) The substitution of some element or group in the naphthalene molecule.

Neither of these methods was fully investigated, but experiments with  $\alpha$ -chlornaphthalene showed that the introduction of chlorine into the naphthalene ring had a marked stabilising influence. Lots of 500 gm. of "allotment" soil plus 15 per cent. of sand were taken and each treated with equimolecular quantities of naphthalene and  $\alpha$ -chlornaphthalene. In addition, two sets were treated with mixtures of naphthalene and  $\alpha$ -chlornaphthalene in equimolecular proportions. The treated soils were then placed together with a number of wireworms in glass jars, which were closed with screw-cap lids. Examinations were made each week when a fresh supply of wireworms was added. The results are given in Table V.

Table V.

*Comparative duration of toxicity of naphthalene and  $\alpha$ -chlornaphthalene in soil.*

Treatment	%	Percentage deaths each week						
		1	2	3	4	5	6	7
Naphthalene	0.05	100	100	0	—	—	—	—
"	0.05	100	100	0	—	—	—	—
"	0.025	100	0	—	—	—	—	—
"	0.025	100	0	—	—	—	—	—
$\alpha$ -Chlornaphthalene	0.064	100	100	100	100	100	100	0
"	0.064	100	100	100	100	100	100	30
"	0.032	100	100	66	75	0	0	—
"	0.032	75	83	66	100	0	0	—
"	0.032	100	100	80	75	50	0	—
Naphthalene and	0.025	100	66	0	0	—	—	—
$\alpha$ -Chlornaphthalene mixture	0.032							
"	"	100	50	0	0	—	—	—
Control (1)	0	0	0	—	—	—	—	—
" (2)	0	0	0	—	—	—	—	—

The results obtained with 0.032 per cent. of  $\alpha$ -chlornaphthalene indicate that it is slower in its toxic action than naphthalene, but the data in Table V clearly demonstrate that it persists in the soil for a greater period of time. It is a matter of surprise to find that the mixture of naphthalene and  $\alpha$ -chlornaphthalene fails in toxicity at the end of the second week and that instead of the  $\alpha$ -chlornaphthalene exercising any stabilising action on the naphthalene, the latter tends to de-stabilise the chlornaphthalene. Although no opportunity presented itself of confirming this result, it will be shown later that the addition of naphthalene to the soil causes, after a few days, a considerable rise in bacterial numbers, and it is not unreasonable to believe that amongst the organisms selected out by the naphthalene and which presumably finally decompose it, there will be variations, and that amongst them certain varieties will be capable of breaking up the  $\alpha$ -chlornaphthalene molecule; the presence of naphthalene in the soil will tend to increase their number and so lead to a more rapid break-up of the chlornaphthalene molecule.

*Determination of the rate of disappearance of naphthalene from soil.*

In view of the fact that the toxicity of naphthalene in the soil only lasted in many cases for a brief period, it was considered advisable to determine its rate of disappearance by chemical methods. Methods for the determination of naphthalene, particularly in coal-gas, have been described. These invariably depend upon the formation of a mole-

cular compound with picric acid, known as naphthalene picrate,  $C_{10}H_8 : C_6H_3N_3O_7$ , a relatively unstable body, but only slightly soluble in water. The reaction of naphthalene with picric acid takes place fairly readily, and the naphthalene can be determined either by direct weighing as naphthalene picrate or, if a standard solution of picric acid has been employed, by titrating the solution of picric acid before and after treatment with standard alkali<sup>1</sup>. Küster<sup>(6)</sup>, Colman and Smith<sup>(1)</sup>, Gair<sup>(2, 3)</sup> and Somerville<sup>(2)</sup> have devised different modes of carrying out the estimation of naphthalene by the employment of this reaction. Küster's method, as modified by Colman and Smith, appeared too complicated and too slow for determining small amounts at intervals of a few hours. The use of relatively large amounts of acetic acid in Gair's method added to the difficulties of estimating by titration with standard alkali. Somerville has suggested the employment of alcohol for the absorption of naphthalene and its subsequent precipitation by a large excess of an aqueous solution of picric acid; a modification of this method was finally adopted and is described on a later page, but in the earlier stages of the work a number of experiments were carried out using aqueous picric acid for purposes of absorption.

*Estimation of naphthalene in soil.* Two methods can be used for isolating naphthalene from the soil. (1) The naphthalened soil can be subjected to a stream of gas, at a moderately high temperature, the naphthalene vapour being absorbed in some suitable solvent or directly precipitated by a solution of picric acid. (2) The soil can be subjected to distillation with steam, and the naphthalene determined in the distillate. Both methods were used as a check upon each other.

(1) *Aeration method.* The naphthalened soil, after being mixed with sand, was aerated in a U-tube, the sand and soil being placed in the limb of the U-tube furthest from the absorption apparatus; the other limb contained glass-wool and above the glass-wool an amount of phosphoric pentoxide was loosely packed to absorb moisture and ammonia. Any carbon dioxide and sulphuretted hydrogen were absorbed by a caustic soda solution in a small all-glass absorber, which could be warmed if any lodgment of naphthalene was noted; the gases then passed by way of a tube, having several constrictions along its length and which acted as a spray trap, to two absorption vessels in series. The absorption vessels were tubular but gradually narrowed towards their lower ends so that the inlet tube only allowed the narrowest

<sup>1</sup> Titration of the picric acid or naphthalene picrate can also be carried out by means of titanous chloride (5).

of margins for the passage of air, a bulb mid-way along the tube permitting of a good deal of splashing without loss of solution. 4–6 c.c. of 0.9 per cent. aqueous picric acid were used for absorbing the naphthalene and at the end of the experiment the volume was brought up to a definite mark on the limb of the absorption vessel, by the addition of a little distilled water. After the experiment the precipitate of naphthalene picrate was centrifuged out and an aliquot part of the clear supernatant liquid was pipetted off and titrated with  $N/50$  or  $N/100$  caustic soda. A few experiments were carried out using a solution of picric acid in water containing 20 per cent. glycerine in the absorption vessel; the glycerine however was observed to introduce a buffering effect on the titration and was finally discarded. Air or naphthalene-free coal-gas, drawn through the apparatus by a water-pump, was used for carrying over the naphthalene. The use of coal-gas was finally discarded as the results obtained did not differ materially from those obtained with air. The U-tube was heated to  $150^{\circ}\text{C}$ . in a glycerine bath, and the whole absorption apparatus protected from draughts by means of an asbestos box, which could be kept at a fairly constant temperature by means of a micro-burner.

Acknowledgments and thanks are due to Major G. G. Hyde, for help given during the early stages of working out a suitable method of estimating naphthalene by the aeration method.

The titration of so highly coloured a solution as that of picric acid presented some difficulties—as in ordinary white light the turning point of the indicator was greatly obscured. It was observed, however, that if the titration were carried out in a light of about the same colour as that of picric acid solution, the latter appeared to the eye nearly colourless, and the end point was quite sharply defined. The titrations were carried out therefore in a room illuminated by means of an electric bulb immersed in a strong solution of picric acid; the addition of a little eosin to the latter rendered the effect of decolorisation still more marked. Brom-cresol purple, phenol or cresol-red were found to be the most suitable indicators.

The method is extremely tedious and requires continuous attention for several hours. It was finally replaced by a method depending on distillation in steam. Fairly constant results however were obtained and these are consistent among themselves and show the same relative order of differences as those obtained by a distillation method, to be described later.

Using 10 gm. of naphthalened garden soil containing 50 mg. of naphthalene per 100 gm. of soil for each test, eight estimations gave

results lying between 38.3 and 41.0 of naphthalene with a mean of 39.3 mg. At a later stage it was found that a greater proportion of the naphthalene could be recovered by using a smaller amount of soil in the tests and increasing the volume of the picric acid solution in the absorbers relative to the amount of naphthalene to be absorbed. The following results were obtained when 5 gm. of soil were aerated instead of 10.

	Added to 5 gm. of soil mg.	Recovered from 5 gm. of soil mg.
1	2.6	2.2
2	2.5	2.32
3	2.5	2.44
4	2.5	2.33
5	2.5	2.36

A control test with untreated soil showed neither concentration nor condensation in the absorbers and the titration figure of the picric acid was unchanged.

*The formation of formaldehyde during the aeration of heated soil.*

During the aeration in the cold of soil fairly rich in humic material, which had been heated to 150° C. for several hours and then allowed to cool, the characteristic odour of formaldehyde was noted. Its presence was confirmed by the phloroglucinol test. In this instance, lime was used in the U-tube and aeration was continued in the cold for much longer than usual. The reaction is of great interest in itself, but as formaldehyde affects picric acid solution, it also indicates that it is advisable not to prolong unduly the aeration of the soil after the naphthalene has been volatilised.

*Experiments with a garden soil and with a soil from a cucumber house.*

2000 gm. of a garden soil and 2000 gm. of a soil, rich in humus, from a cucumber house were each mixed with 1 gm. of finely ground naphthalene, placed in two large bottles, leaving ample air-space above the soil, and closed with cotton-wool plugs. Each day 10 gm. of soil were taken after thorough mixing and the naphthalene determined by the aeration method. At the same time the numbers of bacteria in the garden soil were estimated by Mrs D. J. Matthews, to whom the author wishes to express his thanks. The gelatine plate method was used in these counts. Later work has shown this method to give results of too high an order; absolute numbers are, however, for

# Decomposition of Naphthalene in the Soil

Table VI.

*Rate of disappearance of naphthalene from two soils.*

Day	Bacterial nos. in allotment soil, millions per gm. above control	10 gm. of soil used in aeration.			"Cucumber" soil.		
		"Allotment" soil.			Naphthalene found in mg. per		
		Naphthalene found in mg. per 100 gm. soil			Naphthalene found in mg. per 100 gm. soil		
		(1)	(2)	Mean	(1)	(2)	Mean
1	10	37.8	—	37.8	35.4	38.3	36.8
2	7.5	34.3	—	34.3	30	28.5	29.2
3	2.5	32.4	35.1	33.7	22	26	24
4	20.0	30.9	32.4	31.6	0.44	0.49	0.46
5	—	26.5	27.1	26.8	Trace		
6	800	21.2	23.4	22.3	—	—	—
7	750	4.2	3.9	4.0	—	—	—
8	—	Traces			—	—	—

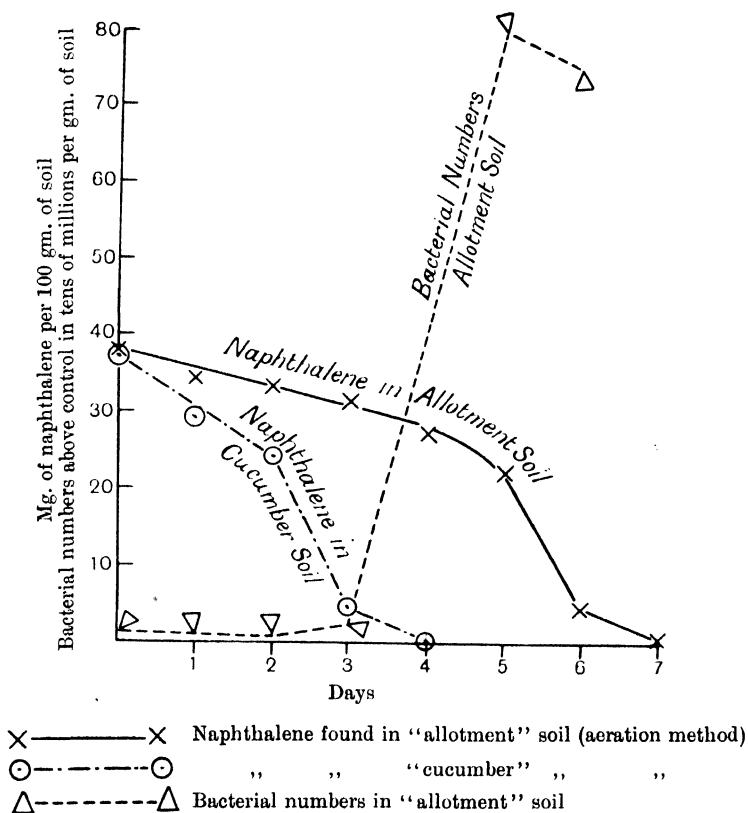


Diagram 1. Decomposition of naphthalene in two soils.

this purpose of less value than relative figures, and the counts are of value and interest as they serve to show that a considerable rise in bacterial numbers took place just prior to and during the period when the rate of disappearance of the naphthalene was greatly accelerated. The data obtained are given in Table VI and the mean values are expressed graphically in Diagram 1.

These data indicate that the rate of disappearance of naphthalene depends entirely upon the type of soil used, the soil richest in organic matter and presumably therefore in microbiological population inducing the more rapid decomposition.

*Decomposition in sealed bottles.*

A further series of experiments was carried out with the "cucumber" soil. Eight lots of 400 gm. of cucumber soil were mixed with 0.2 gm. of powdered naphthalene, placed in separate bottles of 500-600 c.c. capacity and sealed by screw capped lids fitted with rubber bands. From time to time, the bottles were opened, the soil rapidly mixed, the bacterial numbers determined and the naphthalene estimated in 5 gm. of the soil, the remainder of the soil being put back into its bottle and sealed down.

The bacterial numbers were determined in triplicate by Mr D. W. Cutler, to whom the author wishes to express his thanks. The data are given in Table VII and Diagram 2.

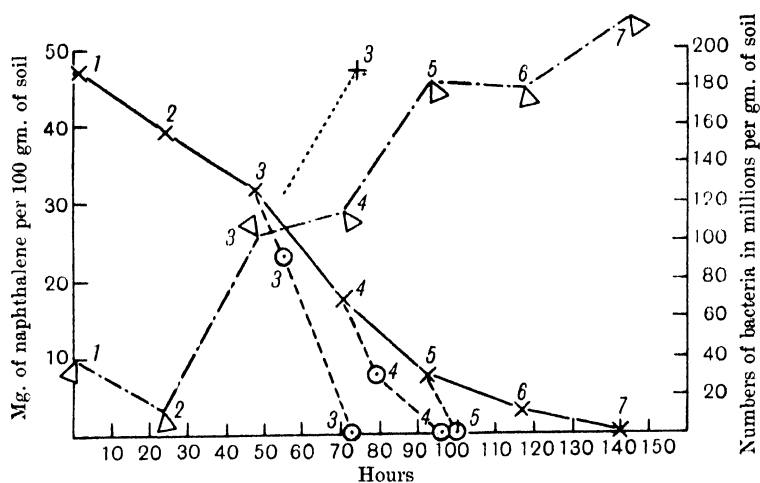
Table VII.

*The disappearance of naphthalene from "cucumber" soil  
(sealed bottle experiment).*

50 mg. naphthalene originally added per 100 gm. of soil.

No. of bottle	Time from mixing (hrs.)	First opening of bottle.			Bacterial nos. Millions per gm. soil. Mean of 3 determinations	Second and third openings of bottle	
		Naphthalene found, mg. per 100 gm. soil				Time from mixing (hrs.)	Naphthalene found, mg. per 100 gm. soil
		1	2	Mean			
1	0.5-0.75	46.6	47.1	46.8	39.3	5.3	43.3
2	23-24	39.6	38.0	38.8	14	29.5	36.4
3	47.5	32.4	31.6	32	102.6	55	(2nd) 22.8
						73	(3rd) None found*
4	70-71	17.6	17.1	17.35	115.6	79.25	(2nd) 7.4
						96	(3rd) None found
5	93-93.5	6.4	8.5	7.5	183.6	100.75	None found
6	117	3.2	—	3.2	179.3	124	None
	143	None	—	None	212	—	—

\* Bacterial nos. rose to 194.3 millions per gm.

*Decomposition of Naphthalene in the Soil*

The numerals 1-7 are the numbers of the bottles

- ×———× Amounts of naphthalene at 1st sampling of each bottle
- „ „ „ 2nd and 3rd sampling of each bottle
- △-.-.-.-△ Bacterial numbers at 1st sampling of each bottle
- .....+ „ „ 2nd sampling of 3rd bottle

Diagram 2. The decomposition of naphthalene in "cucumber" soil (sealed bottles experiment).

It was noted that after any bottle had been opened, and the soil stirred, the rate of disappearance of the naphthalene was apparently accelerated; therefore, in addition to the determination of the naphthalene immediately after the first opening, the same bottle was reopened a few hours later, and again 24 hours after the first opening, and the amount of naphthalene again determined. An inspection of Table VII and Diagram 2 indicates that the opening of the bottle and the re-mixing of the soil materially expedites the disappearance of the naphthalene from the soil, and also causes a material increase in bacterial numbers. As little or no naphthalene could have volatilised in the brief time required for sampling, this simple operation must in some way have acted as a stimulus to the factor determining decomposition.

The preliminary fall in bacterial numbers indicates a selective toxic action of the naphthalene on part of the bacterial flora; the subsequent large rise shows that certain types of organisms are either capable of using naphthalene as a source of energy, or are stimulated to greater activity by its presence.

In all probability the estimations of naphthalene as carried out by the aeration method are valid in so far as they indicate the relative rates of disappearance of naphthalene from soil. The method as employed, however, is so tedious and difficult to operate with success that it was considered advisable to check the results by another means. Somerville's method(2) indicates that alcohol can be used in the absorption vessels without interfering materially with the precipitation of naphthalene picrate, provided it is sufficiently dilute and a large excess of picric acid is present. It was therefore decided to use an alcoholic solution of picric acid for absorbing the naphthalene and to distil the latter from the soil into the alcoholic picric acid.

(2) *Estimation of naphthalene by steam distillation.* 100 gm. of naphthalened soil were acidified with the smallest amount of phosphoric acid necessary, and distilled in steam.

The outlet of the distillation flask was fitted with a spray trap through which the steam was passed into a Matthews ammonia absorption tube(7) containing 25 c.c. of a 5 per cent. alcoholic solution of picric acid, the absorption tube being allowed to get warmed by the passage of the steam in order to ensure complete reaction between the picric acid and naphthalene which distilled over very rapidly. A guard tube containing an aqueous picric acid solution was attached in series to the absorption tube to prevent loss of naphthalene. As soon as the greater portion of the naphthalene had passed over, both main absorption tube and guard tube were cooled. After the completion of the distillation, which took about half to three-quarters of an hour, the absorbers were aerated for 20-30 minutes while being cooled. Both absorbers were washed out into a 250 c.c. flask with an accurately measured quantity of aqueous picric acid solution and then with a little distilled water and the flask filled up to the mark. 100 c.c. of the liquid were then filtered and titrated with standard caustic soda. The titration was again carried out in a yellow or orange coloured light obtained in the way previously described. For amounts of naphthalene less than 10 mg. per 100 gm. of soil a small absorber was used and a correspondingly smaller amount of alcoholic picric acid (the alcohol present in the graduated flask should not exceed 10 per cent. in amount and should preferably be less). If  $\text{H}_2\text{S}$  or  $\text{CO}_2$  is liberated in large amounts from the soil a second distillation flask containing caustic soda solution may be interposed between the first distillation flask and the absorber. This was not found necessary with the soil used and a control distillation of 100 gm. of the soil showed no effect upon the titration

of the picric acid. The following results were obtained in test trials:

	Naphthalene added to 100 gm. of soil mg.	Naphthalene found in 100 gm. of soil mg.
(1)	50	47.7
(2)	50	49.0
(3)	50	49.66
(4)	1.1	1.5
(5)	1.2	1.23
(7) Control	0	0

The method is less accurate for the determination of amounts of naphthalene less than 5 mg. per 100 gm. of soil than for the higher concentrations.

*Experiments with repeated doses of naphthalene.*

These experiments were set up to ascertain whether, when naphthalene had been added to the soil and allowed to disappear, and the soil again re-treated, subsequent doses would be decomposed more rapidly than the first, as would be expected if the decomposition were due to the micro-organic population in the soil.

Lots of 100 gm. of "cucumber" soil were therefore treated with 50 mg. of naphthalene and placed in 1000 c.c. flasks fitted with cotton-wool plugs. The rate of disappearance was determined and when the naphthalene content of the first series had been reduced to a minute amount (in 96 hours), a further quantity of 50 mg. was added to each of the remaining flasks and well mixed with the soil. The naphthalene now disappeared

Table VIII.

*The effect of re-adding naphthalene to soil from which it had disappeared.*

100 gm. of "cucumber" soil used for each test. Concentration of naphthalene at beginning 50 mg. per 100 gm. of soil.

First treatment		Second treatment		Third treatment	
Time	Naphthalene found (mg.)	Time (hrs.)	Naphthalene found (mg.)	Time (hrs.)	Naphthalene found (mg.)
4 hrs. 25 mins.	49.66	0	50 mg. (added)	0	50 mg. (added)
18 " 50 "	47.34	24	1.0	3	20.2-21.2
45 " "	36.76	—	—	5	8.7
72 " "	2.7	—	—	8	2.8
77 " "	1.0	—	—	11	2.3
96 " "	Mere trace				

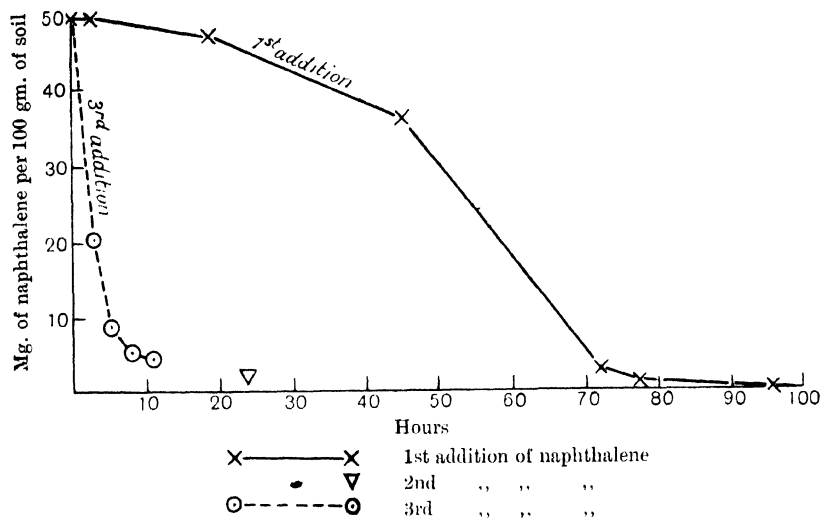


Diagram 3. Effect of re-adding naphthalene to soil ("cucumber" soil).

in 24 hours. A third addition of naphthalene was then made and at the end of three, five, eight and eleven hours, 20 c.c. of a solution of mercuric chloride was added in order to stop the reaction and the naphthalene remaining was estimated. The data which are set out in Table VIII and Diagram 3 clearly show that doses subsequent to the first one disappear from the soil at a much greater rate.

#### *Experiments with sterile soil.*

In view of the acceleration in the rate of decomposition of naphthalene on subsequent re-additions to naphthalened soil, experiments were made to test the rate of disappearance from sterile soil. Six lots of 100 gm. of soil from a cucumber house were placed in flasks of 1000 c.c. volume which were plugged with cotton-wool stoppers, so rolled that the flasks could just be supported when held by the wool. The soil was sterilised by autoclaving at 15-20 lb. pressure and then allowed to cool for 24 hours. Pure and sterile naphthalene was then prepared by flooding it in a round-bottomed flask with absolute alcohol, the alcohol being subsequently evaporated off *in vacuo*. 0.05 gm. lots (50 mg.) of the naphthalene were weighed out on sterile watch glasses and rapidly transferred to the flasks, well mixed with the soil and the flasks laid on their sides in a cellar which was known to keep at a fairly constant temperature for prolonged periods.

A similar set using unsterilised soil was set up at the same time. At intervals the naphthalene in each flask was determined by the distillation method. The results are set out in Table IX and Diagram 4.

Table IX.

*Decomposition of naphthalene and chlornaphthalene. Experiments with sterile and non-sterile soil (from cucumber house).*

Time	Non-steril soil. Naphthalene found mg. per 100 gm. soil	Time	Sterile soil. Naphthalene found mg. per 100 gm. soil	Time (hrs.)	Non-sterile soil. Chlornaphtha- lene found. mg. per 100 gm. soil
—	50 (added)	—	50 (added)	—	0.05 c.c. (added)
4 hrs. 25 mins.	49.66	24 hrs. 10 mins.	49.55	—	50.1
18 „ 50 „	47.34	96 „	43.8	47	48.4
45 „	36.76	240 „	43.4	78	45.0
72 „	2.7	—	—	172	41.7
77 „	1.0	—	—	214	42.8
96 „	Mere trace	—	—	362	33.4
				526	30.13

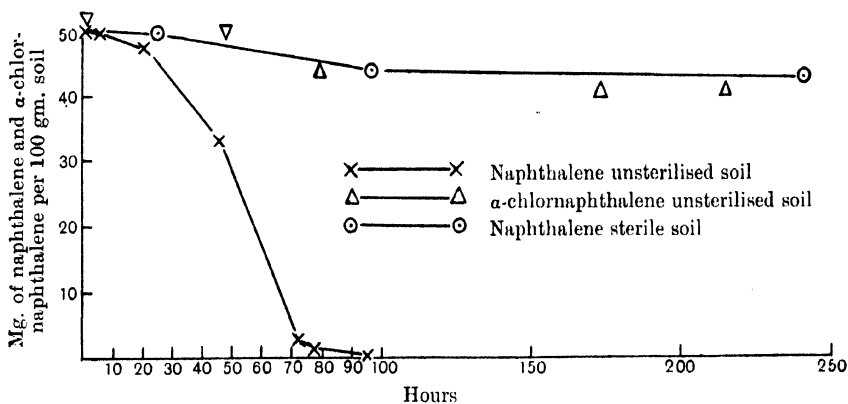


Diagram 4. Rate of disappearance of naphthalene and chlornaphthalene from "cucumber" soil

Inspection of Table IX and Diagram 4 confirms the deductions drawn from all the data given, that the disappearance of naphthalene is due to the micro-organic population of the soil. There is some loss of naphthalene from sterile soil, but as the cotton-wool plugs smelt slightly of the chemical, this was probably due to volatilisation.

*Rate of disappearance of chlornaphthalene from soil.*

In view of the rapid decomposition of naphthalene, experiments were made to ascertain whether it could be stabilised. It was considered that substitution of suitable groups in the naphthalene ring might be effective. Preliminary toxicity trials indicated that the substitution of a chlorine atom in the naphthalene molecule had a stabilising action (p. 66) and the rate of disappearance of  $\alpha$ -chlornaphthalene from soil treated with this chemical was therefore determined. The experiments were carried out at the same time as the experiments set out in Table IX. Approximately 0.05 c.c. of redistilled  $\alpha$ -chlornaphthalene was pipetted by means of a capillary pipette into each of six 1000 c.c. flasks containing 100 gm. of non-sterile "cucumber" soil, the flasks plugged with cotton-wool and laid on their sides in a cellar. From time to time the amount of chlornaphthalene was determined by distilling in steam into standard alcoholic picric acid as in the case of the determination of naphthalene. Control tests gave a recovery of (1) 51.1 and (2) 49.1 mg. of  $\alpha$ -chlornaphthalene when the estimation was made shortly after mixing. The method is not quite as satisfactory for chlornaphthalene as for naphthalene; whether this is due to the chlornaphthalene not being quite pure or to a lower quantitative efficiency in its determination was not ascertained. The results, however, are comparable among themselves as the tests were carried out in as constant a way as possible. The data obtained are given in Table IX and Diagram 4. They indicate that there is a slight loss with time, but that chlornaphthalene is as stable in non-sterile soil as naphthalene in sterile soil, and while naphthalene disappears from "cucumber" soil almost entirely in 77 hours, chlornaphthalene shows only a comparatively slight diminution in amount in a period of 526 hours.

*Nephelometric method of estimation.*

Neither of the preceding methods could be regarded as suitable for estimating naphthalene in minute amounts. Some experiments were made to ascertain whether the nephelometer could be employed for this purpose, although these were not carried far enough to be employed upon naphthalened soil. It was found that by using a solution of picric acid containing 0.8 per cent. agar and 0.1 per cent. saponin and pouring in a solution of naphthalene in alcohol, the picrate of naphthalene was precipitated in a very finely divided form. The crystals, however, had a

tendency to grow or to aggregate, but this took place comparatively slowly. It is probable that some such method would be adaptable for tracing out the lower portion of the decomposition curves.

*Erosion of a crystal of naphthalene.*

An attempt was made to demonstrate the mode of attack upon a crystal of naphthalene. A modified Lipmann's medium was prepared containing naphthalene in place of dextrose, and 1 c.c. of a soil solution prepared from soil in which naphthalene had already been decomposed was pipetted into this medium and one subculture made in the same



Fig. 1. Erosion of naphthalene crystal (highly magnified).

medium. A small micro-petri dish was prepared by sealing by means of sodium silicate a small ring on to a microscope slide, and a slightly larger ring was sealed to a large cover slip. Both were sterilised and a few particles of recrystallised naphthalene were scattered on the cover-slip within the ring. One drop of the second subculture was allowed to fall on the naphthalene and a little melted nutrient agar was finally poured in, forming on cooling a thin semi-rigid film holding some of the naphthalene against the cover-slip; the cover-slip and ring were then placed over the ring on the microscope slide, allowed to stand at room temperature and examined microscopically each day. In three days

the edges of certain crystals showed very slight erosion. One of these was marked and after a week photographed. At the beginning of the experiment the edge *AB* was straight, but the illustration (Fig. 1) demonstrates that in a period of eight days it had been eroded into small bays. This serrating effect is probably due to unevenness in the decomposition of the crystal layers, which permitted attack at certain favourable situations along the crystal edges. In other cases erosion takes place more evenly but invariably bacteria were observed exhibiting strong Brownian movement in the medium close to the crystal edges.

All the evidence educed in the preceding pages points to one or more micro-organisms being the active agents in the decomposition of naphthalene in the soil. The biological aspects of the work were investigated by Gray and Thornton<sup>(1)</sup> who have demonstrated the wide geographical distribution of soils containing bacteria capable of using cyclic hydrocarbons, including naphthalene, as sources of energy. A number of these organisms have been isolated and described.

#### SUMMARY.

1. The insecticidal action of naphthalene and its duration in the soil have been studied.

2. When naphthalene is incorporated thoroughly with soil it shows a fairly potent toxic action on wireworms; uneven distribution lessens its efficiency as, owing to its low vapour pressure and consequent slow spread, it produces only a small zone of toxic action.

3. Naphthalene is slow in toxic action, taking three or four days to kill wireworms, as a consequence of which and of its repellent action to insects, if the chemical be unevenly distributed in the soil insects tend to move away from positions where toxic action would be exerted.

4. The persistence of the toxic action depends upon the soil type. In soils rich in organic matter, toxicity disappears more rapidly than in soils less rich in organic matter. Toxicity persists longer in sterile soils and in sand than in unsterilised soils, and in dry than in moist soils.

5. The rate of disappearance of naphthalene from soil has been determined. It depends very little upon volatilisation but almost entirely upon some factor inherent in the soil, which is more active in soils rich in organic matter than those poor in organic matter, and in unsterilised soils than in sterile soils.

6. Second and third doses of naphthalene added to the soil, when the first has disappeared, are decomposed more rapidly than the first dose.

7. The bacterial numbers of the soil are at first decreased by the addition of naphthalene, but there is a rapid rise during the period when acceleration in the rate of decomposition of the naphthalene is taking place. All the evidence indicates that the loss of naphthalene from the soil is mainly due to bacterial decomposition.

8. Experiments in sealed bottles indicate that the opening of the bottle and mixing of the sample expedite the disappearance of naphthalene from the soil.

9. The toxicity of  $\alpha$ -chlornaphthalene persists for a longer time in soils than naphthalene, and is decomposed at a slower rate. An admixture of naphthalene appears to induce a more rapid disappearance of the toxicity of the chlornaphthalene.  $\alpha$ -Chlornaphthalene is more toxic to plants than naphthalene.

10. Methods of estimating naphthalene are described. They depend on formation of naphthalene picrate. Picric acid can be more readily titrated by alkali in orange and yellow coloured light than in white light.

11. It was noted in several tests that the prolonged aeration of soils which had been heated and allowed to cool gave rise to formaldehyde.

#### REFERENCES.

- (1) COLMAN, H. G. and SMITH, J. F. (1900). *Journ. Soc. Chem. Ind.* xix, 128.
- (2) GAIR, G. J. D. (1905). *Ibid.* xxiv, 1279.
- (3) — (1907). *Ibid.* xxvii, 1263.
- (4) GRAY, P. H. and THORNTON, H. G. (1927). *Centr. f. Bakt. Abt.* ii, LXXIII, 74.
- (5) KNECHT, E. and HIBBERT, E. (1918). *New Reduction Methods of Volumetric Analysis*. Longmans, Green, p. 75.
- (6) KÜSTER, F. W. (1894). *Ber. Deut. Chem. Ges.* xxvii, 1101.
- (7) MATTHEWS, D. J. (1920). *Journ. Agric. Sci.* x, 72.
- (8) TATTERSFIELD, F. and ROBERTS, A. W. R. (1920). *Journ. Agric. Sci.* x, 199.

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# ENGLISH-GROWN PYRETHRUM AS AN INSECTICIDE. I

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## INTRODUCTION.

THE use of certain species of pyrethrum (*Chrysanthemum*) for insecticidal purposes has been known for a considerable period of time; Dalmatian, Persian and Caucasian insect powders consist of the ground flowers, respectively, of *Chrysanthemum cinerariaefolium* Trev., *C. coccineum* Willd., and *C. marschallii* Ascher (syn. *Pyrethrum roseum* Bieb.)<sup>1</sup>, though there appears to be some doubt as to whether the two latter are distinct species.

*Chrysanthemum cinerariaefolium* has been grown in Dalmatia and Japan for a number of years and its cultivation has steadily spread to almost all parts of the world. Excellent accounts of the history, character and cultivation of this plant, with a description of some of the work carried out to ascertain the nature and composition of its insecticidal principle and means for detecting its adulteration are given in Juillet's

<sup>1</sup> The Insecticide and Fungicide Board of the United States Department of Agriculture recognises these three species as those from which genuine insect powder is made.

monograph<sup>(5)</sup> and in the paper of MacDonnell, Roark and Keenan<sup>(6)</sup>. Both of these works contain full bibliographies. It would therefore serve no useful purpose to refer here at any length to the literature of the subject, and the reader interested is referred to the above publications.

The cultivation of pyrethrum has become a fairly extensive practice in many countries. It is a matter of surprise therefore to find that only spasmodic and half-hearted attempts to raise this valuable plant have been undertaken in this country. It is recognised that the economic possibility of growing pyrethrum will depend upon the yield, upon whether it can be kept free from disease and whether, under our climatic conditions, the crop can be harvested and dried at an economic cost. The present investigation was undertaken to study the growing of pyrethrum for a period of years in this country with particular reference to the type of soil most suitable for its growth, and to the possibility of the occurrence of variations in toxicity in the flowers grown in different localities. Other points requiring study were the keeping qualities, the variation, if any, of toxicity with size of flower-head, and whether or no any specificity of toxic action is shown.

The authors wish to record their indebtedness to the authorities of the following Stations for their kind collaboration in growing plots of pyrethrum: Department of Agricultural Education, Worcester; Research Station, Long Ashton; Isle of Ely Demonstration Plot; University College, Reading; South-Eastern Agricultural College, Wye; East Malling Research Station; The Horticultural College, Swanley; Royal Horticultural Society, Wisley; The Farm Institute, Sparsholt, Hants; Seale Hayne Agricultural College, Newton Abbot; University College of Wales, Aberystwyth; Experimental Station, Scilly Is..

#### EXPERIMENTAL CULTIVATION OF PYRETHRUM.

A preliminary report on the experimental cultivation of pyrethrum in England appeared in the *Journal* of the Ministry of Agriculture<sup>(3)</sup> and dealt with the results of the work up to the close of 1926. The origin of the experiments was there described, and it will be sufficient for the purposes of the present paper if a brief summary of the earlier work be given.

*Origin of seed.* Thanks to the generosity of M. Vaysière, of the Station Entomologique de Paris, and of Messrs Joensson and Co., of Kobe, seed of pyrethrum, *Chrysanthemum cinerariaefolium*, both of European and Japanese origin, was obtained in 1924 and 1925. The European seed was produced at the Station Viticole at Lausanne, where experiments in the

cultivation of pyrethrum have been in progress for some years. The Japanese seed was of the standard commercial quality used for growing crops in Japan, where pyrethrum production has been carried out on a large scale for a much longer period. Two different races of pyrethrum were thus involved in the experiments, and, as was to be expected, considerable differences between them were discovered. The plants from the Swiss seed showed evidence of selective propagation in the relative uniformity both of habit of growth and of cropping, whereas the plants from Japanese seed were very diverse in both respects and suggested that no attention is given in Japan to the production of special strains of the plant. Although the Swiss plants were more uniform in character than the Japanese, they nevertheless showed considerable variation among themselves, and if habit of growth should prove to be correlated with toxicity of flowers, the strain should be capable of much further improvement at the hands of the plant breeder.

As soon as seed of the two strains was available, the kind co-operation of the authorities of a number of experiment stations was secured, and finally eleven plots of Swiss pyrethrum and nine of Japanese were planted. Owing to the fact that the Swiss seed was small in amount and arrived earlier than the Japanese, while the latter germinated badly, it was not possible to arrange that at each station there should be plots of both Japanese and Swiss plants, but sufficient stations are growing both races to enable a fair comparison to be obtained.

*Soil.* European accounts of pyrethrum growing invariably point out that the plant when growing wild is found on the poor calcareous soils of the Dalmatian region, and not unnaturally its cultivation on such soils is usually recommended. It is less clear, however, that the range of soils capable of growing good pyrethrum has been sufficiently explored, and therefore, in initiating the present experiments, it seemed wise to include a variety of soil types. In the present paper insecticidal tests are described with pyrethrum from different plots and the type of soil on these plots is briefly indicated as follows:

Harpenden ...	...	...	...	Clay with flints
East Malling ...	...	...	...	Light loam over greensand
Seale Hayne (Newton Abbot) ...	...	...	...	A sticky Devonian sandstone
Scilly Is. (St Mary's) ...	...	...	...	Light sandy granite soil
Sparsholt ...	...	...	...	Light calcareous loam
Swanley ...	...	...	...	Light thin loam on chalk
Wye ...	...	...	...	Chalk (patches of light loam)

The above list shows that a variety of soil textures have been obtained, although it is probable that so far as these particular stations

are concerned most of them show no marked lime deficiency (with the possible exception of the Scilly Is. and Newton Abbot). The subject will be discussed further when the results of tests with the 1927 crop are available.

*Cultivation.* Pyrethrum is a perennial which under English conditions requires rather more than a year before it comes into full bearing. Seed sowing may thus take place either in the autumn or spring, and the first crop will be secured in the second summer after sowing. Under Harpenden conditions, to which the following notes chiefly refer, the best results were obtained by sowing in autumn, the young plants being wintered in cold frames and planted out in the following spring; sowing in the open proved less successful, and since the plants gave little trouble in the seed pans or when pricked out in cold frames, it seems undesirable. In planting out, the French instructions were followed approximately and the young plants were set out at a distance of 18 in.  $\times$  18 in. By the following autumn the plants were beginning to touch and when the first crop was taken the ground was completely covered, so that small annual weeds were entirely suppressed and it was only necessary to remove by hand occasional large weeds, such as sow thistle and the larger grasses, which had succeeded in rising above the crop. In the winter there is considerable dying off of the old foliage, especially the outer leaves, which gives the crop a rather poor appearance at the end of winter, but as spring advances new shoots are developed and a healthy foliage is rapidly regained. It would seem that the pyrethrum plants are hardy under English conditions as they have withstood, without apparently suffering, the winters of 1925-26 and 1926-27, which were fairly representative; and this was the case even under very trying soil conditions, such as where the crop was planted in heavy wet clay. In regard to the duration of a plantation under English conditions, the oldest plot is one from Swiss seed, which was planted out in April 1925. Since then two harvests have been taken and in general appearance the plot looks good for at least another harvest. In France, a plantation is expected to last from eight to nine years, but it is improbable that so long a duration would be obtained in England.

*Harvest.* Although both leaves and flower-stems contain the insecticidal principles in small quantities, it is the flower which is chiefly of value. There is, however, no uniformity of opinion as to the exact stage at which the flower should be cut. In commercial circles it has become the established convention to regard as good samples only those containing flower buds in the almost closed condition. The results of certain

investigations in France and elsewhere suggest, however, that this convention is without foundation, open flowers being as toxic as almost closed buds. Since a considerably greater weight of crop can be obtained from a given area if harvest is delayed until the flowers are nearly open, it was considered desirable to test flowers cut at different stages, and details of this experiment appear elsewhere in this paper. So far as the majority of plots was concerned, however, an endeavour was made to cut flowers when the petals had fully developed but before they had flattened out. Local conditions—weather, labour, etc.—interfered with this endeavour in several cases, and as a result the samples from the different stations showed considerable variation—some consisting of almost closed buds as approved commercially and others of practically fully open flowers. (The experiment referred to above will show that except in so far as the total weight of crop is concerned this variation appears to be more or less immaterial.)

Harvesting consists of cutting the flowers with a convenient length of stalk (about 8 in.) and then removing the flower-heads either before or after drying—which is carried out by exposing the crop, spread in a thin layer, first out-of-doors (if there should happen to be any sunshine) and subsequently in a ventilated building. After drying for about six weeks the flower-heads can be safely stored in metal bins or tins. In regard to yield, considerable variation is shown by the different plots, but it is not proposed to discuss this subject, or indeed the economic possibilities of pyrethrum growing, in any detail until more information is available. It may, however, be of interest to mention that at Harpenden 1 rod of Swiss pyrethrum yielded 5 lb. of dried flowers in 1926 and 2 lb. 12 oz. in 1927 when cropped for the second time, whereas in 1927 1 rod cropping for the first time gave 3 lb. 5 oz. The average yield of all “Swiss plots” in 1927 was 3 lb. 14 oz. per rod. A conservative estimate of the yield in France is given as 2½ lb. (approximately) per rod, and it is clear that English yields compare with this not unfavourably.

#### EXPERIMENTS WITH *APHIS RUMICIS* L. (BLACK BEAN APHIS).

The method used for the determination of the toxicity values of plant extracts to *A. rumicis* has already been described (10, 11). The essential features are the preparation of the extracts in a constant way, the preparation of the different concentrations of the extracts by dilution with an aqueous solution of saponin or soap, the spraying of the dilutions upon the insects under constant conditions, and the subsequent

classification of the effects under the headings "unaffected," "slightly affected," "moribund" and "dead."

A slight departure from the usual method of preparing the extracts was made, owing to the fact that the samples of pyrethrum flowers were not ground to the same condition of fineness as many of the other plant materials previously investigated. Instead, therefore, of soaking the ground flowers in absolute alcohol and filtering through muslin, the flowers were soaked in a known volume of alcohol for several days, with repeated shaking, allowed to settle and the clear supernatant liquid diluted. Throughout these experiments a 0.5 per cent. solution of saponin in water was used for dilution.

In its effects upon *Aphis rumicis*, pyrethrum is in a class by itself; its action is extremely rapid, producing a state of profound narcosis, which at the higher concentrations results in death; at lower concentrations, the narcotic action may wear off and after a period of time, which appears to depend upon meteorological conditions, the insects often partially and sometimes wholly recover from its effects. This necessitates examination of the insects for several days after spraying and renders classification under the headings "moribund" and "dead" somewhat difficult. It must therefore be understood that under these two headings are collected those sprayed insects which under the conditions of our

Table I.

*Toxicity of pyrethrum to A. rumicis.*

(Showing recovery with time.)

[N = not affected. S = slightly affected. M = moribund. D = apparently dead.]

Derivation of seed and part of plant	Concentration in terms of part of plant gm./100 c.c.	After 24 hours				After 48 hours				After 72 hours			
		N	S	M	D	N	S	M	D	N	S	M	D
Flowers from Swiss seed, Harpenden grown	0.25	—	—	—	10	—	—	—	10	—	—	—	10
	0.1	—	—	2	8	—	—	4	6	—	1	4	5
	0.05	—	—	3	7	—	3	6	1	7	1	1	1
	0.025	—	1	6	3	6	1	3	—	7	1	2	—
Stalks from Swiss seed, Harpenden grown	5.0	—	—	—	10	—	—	—	10	—	—	4	6
	2.5	—	—	—	10	—	—	3	7	—	3	3	4
	1.0	—	3	5	2	4	3	2	1	4	1	2	3
	0.5	4	1	3	2	4	3	—	3	4	1	2	3
Flowers from Japanese seed, Harpenden grown	0.5	—	—	—	10	—	—	—	10	—	—	—	10
	0.25	—	—	—	10	—	1	5	4	—	2	2	6
	0.1	—	—	3	7	—	3	6	1	—	4	2	4
	0.05	—	2	3	5	6	1	2	1	6	1	—	3
Control 1	0.5 % saponin	10	—	—	—	10	—	—	—	7	2	—	1
Control 2	0.5 % saponin	10	—	—	—	10	—	—	—	8	1	—	1

experiments were apparently moribund or dead after being kept for 2 or 3 days. The technique adopted does not allow of observations being continued much longer than this, because some of the unsprayed control insects then normally begin to show signs of failing.

Some examples of experiments showing this tendency towards recovery (which has only been observed by us with a few materials other than pyrethrum) are given in Table I.

In the subsequent tables, we have, for ease of comparison, expressed the moribund and dead together as a percentage of the number of insects sprayed, but owing to the difficulty of classification referred to, we do not lay stress on this figure as an accurate numerical estimate of the toxicities of the various extracts. The detailed figures in the tables form the most reliable basis of comparison.

The observed tendency towards recovery is not a property which invalidates the practical use of pyrethrum. It must be taken into account in detailed experiments on the comparative toxicities of different samples; but, under practical conditions, higher concentrations than the minima giving 100 per cent. "moribund" and "dead" under the conditions of our experiments would be employed. As will be seen, our results demonstrate that pyrethrum flowers have a very high toxicity indeed to certain insects.

#### *Toxicity of pyrethrum grown at different centres.*

The air-dried samples received from the various centres were ground, extracted with alcohol and diluted to known concentrations (expressed in the tables as percentages of the plant material) and sprayed upon adult wingless females of *A. rumicis*, reared under standard conditions, insects of the same size and age only being used. As in previous experiments, 10 insects were sprayed at a time. Control tests were carried out with 0.5 per cent. aqueous solutions of saponin and dilutions of absolute alcohol in 0.5 per cent. aqueous solutions of saponin. Alcohol is not toxic at concentrations considerably higher than those used in the dilutions of the pyrethrum extracts. After spraying, the insects were kept under observation until the controls showed signs of failing. The results obtained with the flowers grown in 1926 at different stations are set out in Table II. The figures for a sample of flowers grown in France are also included, the tests in each case being carried out in 1927 (June to August). The moisture content of all samples tested was determined by drying in an electrically-heated drying oven at a temperature of 104° C.

Table II.

*Toxicity of pyrethrum flowers grown at different stations to A. rumicis.*  
(Harvest 1926: tested 1927.)

[N = not affected. S = slightly affected. M = moribund. D = apparently dead.]

Station	Loss on drying at 104° C. %	Concentration in terms of part of plant gm./100 c.c.	N %	S %	M %	D %	M & D %
Harpenden from Swiss seed	14.8	0.5	—	—	—	100	100
		0.25	—	—	10	90	100
		0.1	15	10	20	55	75
		0.05	70	10	5	15	20
		0.025	70	10	20	—	20
Seale Hayne from Swiss seed	15.1	0.5	—	—	—	100	100
		0.25	—	—	—	100	100
		0.1	—	30	30	40	70
		0.05	40	40	20	—	20
Sparsholt from Swiss seed	14.2	0.5	—	—	—	100	100
		0.25	—	—	15	85	100
		0.1	25	35	30	10	40
		0.05	65	20	10	5	15
		0.025	80	20	—	—	—
Scilly Isles from Swiss seed	14.8	0.5	—	—	—	100	100
		0.25	—	—	—	100	100
		0.1	—	—	40	60	100
		0.05	—	10	75	15	90
		0.025	—	40	60	—	60
Swanley from Swiss seed	14.2	0.5	—	—	—	100	100
		0.25	—	—	5	95	100
		0.1	—	—	15	85	100
		0.05	—	10	60	30	90
		0.025	80	20	—	—	—
Wye from Swiss seed	14.3	0.5	—	—	—	100	100
		0.25	—	10	5	85	90
		0.1	20	5	30	45	75
		0.05	65	15	10	10	20
French grown	14.5	0.5	—	—	—	100	100
		0.25	—	—	—	100	100
		0.1	—	—	10	90	100
		0.05	—	—	20	80	100
		0.025	60	—	30	10	40
Harpenden from Japanese seed	15.2	0.5	—	—	—	100	100
		0.25	—	10	50	40	90
		0.1	—	30	60	10	70
		0.05	60	10	20	10	30
Sparsholt from Japanese seed	13.6	0.5	—	—	70	30	100
		0.25	—	—	90	10	100
		0.1	—	30	60	10	70
		0.05	70	10	20	—	20
Swanley from Japanese seed Dried at 50–65° F.	13.4	0.5	—	—	—	100	100
		0.25	—	10	10	80	90
		0.1	10	10	50	30	80
		0.05	50	40	10	—	10
East Malling from Japanese seed	14.8	0.5	—	—	—	100	100
		0.25	—	—	70	30	100
		0.1	60	10	30	—	30
		0.05	70	30	—	—	—
Wye from Japanese seed	13.2	0.5	—	—	30	70	100
		0.25	—	—	10	90	100
		0.1	60	10	—	30	30

Inspection of Table II shows that the samples grown from Swiss seed at Harpenden, Wye, Sparsholt and Seale Hayne have about the same toxicity, failing to kill 90-100 per cent. of the insects at a concentration rather less than 0.25 per cent. The samples grown at Swanley and in the Scilly Isles and the sample derived from France are apparently somewhat more effective. This result is in all probability significant for these samples under the conditions of our experiments, but it is improbable that small differences of the order found would be detectable in practice. For practical purposes all the samples may be considered to have approximately the same toxicity to *A. rumicis*.

The results obtained with the flowers grown from Japanese seed at the various stations are also all of the same order and show them to be about as toxic as samples grown from Swiss seed. The Japanese flowers grown at Swanley appear to be slightly less effective than the Swiss flowers grown at that station, but again this may be true only for these samples and other things being equal, is hardly of practical significance.

Samples of flowers grown at Harpenden, Seale Hayne, in the Scilly Isles, and in France were also tested on young larvae of the Vapourer Moth (*Orgyia antiqua* L.) which are very sensitive to the action of pyrethrum extract (see p. 440). No differences between the four samples were detectable, all the larvae (10 in each test) being almost immediately killed by spraying with concentrations of 1.0, 0.5 and 0.25 per cent.

*1927 harvest.* An opportunity occurred in 1927 to test flowers from a few of the stations shortly after harvesting. On the whole, the results are closely similar to those obtained for the 1926 harvest; the samples of Japanese flowers grown at Wye would appear to be rather more toxic than a sample from the same plot in 1926. The Swanley (Swiss) flowers again show a slightly higher toxicity than the other samples.

#### *Effect of stage of development of the flowers on their toxicity.*

It appears to be a common practice amongst certain buyers of pyrethrum flowers to prefer those in which the flower is not completely open, as it is said that adulteration is then more readily detected. Juillet<sup>(5)</sup> in his monograph (p. 110) adverts to this practice, and states that in commerce great importance is attached to the degree of openness of the flower, the "closed" buds being regarded as much more effective than the "half-closed," which again are supposed to be superior to the open flowers. Juillet considers this view unsound and quotes Faes<sup>(2)</sup> and Passerini<sup>(7)</sup> as having demonstrated the "open" flowers to be superior to the "closed" on the one hand, and to the over-blown on the other.

In view of the commercial importance of selecting a right time for harvesting, a number of flower heads at different stages of development were chosen from the same rows of a bed bearing a crop of flowers grown from Swiss seed at Harpenden. The stages at which they were taken are almost identical with those shown in the frontispiece of Juillet's monograph, except that the "closed" buds were beginning to show yellowish green petals.

The stages at which the samples were taken may be described as follows:

1. Closed buds.
2. Buds beginning to show white petals.
3. Half-open flowers.
4. Fully open flowers.
5. Very fully open flowers, *i.e.* had been fully open for some days.
6. Over-blown flowers.

The last two samples were taken from an adjoining bed.

Samples 1-4 were dried at ordinary temperature in the shade; sample 5 was treated in three different ways to be described later; and sample 6 was dried at 50° C. in a drying room. All the samples with the exception of the over-blown flowers were tested on the same day.

The results are given in Table III.

Taking into account the difficulty of evaluating the "moribund," the results obtained clearly indicate that little difference exists between the toxicity of the different samples, weight for weight; the differences observed cannot be regarded as outside the error of the experiment. The views of Juillet, Faes and Passerini are confirmed and there is no warrant for evaluating pyrethrum upon a basis of the degree of opening of the flowers. There are however two points of considerable importance to the grower disclosed by these tests. (1) The crop yields progressively increase in weight with the degree of opening of the flowers; thus the weight of the air-dried flower-heads per hundred is in the following order: Closed, 8.8 gm.; slightly open, 14.0 gm.; half-open, 14.75 gm.; fully open, 20.5 gm.; very fully open, 21.6 gm. By taking the crop in the closed stage, there is a loss in actual yield of insecticide per unit area of nearly 60 per cent., and by taking in the half-closed state, of over 25 per cent., as compared with the yield when the crop is taken when the flowers are fully open<sup>1</sup>. (2) There is little advantage to be obtained in taking the flowers beyond the fully open state as the over-blown flowers

<sup>1</sup> The losses would not be so great under practical conditions since it is not feasible to harvest the crop with all the flowers at precisely the same stage of development.

show no superiority in toxicity; indeed, the sample dealt with by us, shed the disc corollae so readily that no determination of the weight of the dry flower-heads could be made.

Table III.

*Effect of degree of development and method of after-treatment of pyrethrum flowers on toxicity to A. rumicis.*

(Grown at Harpenden from Swiss seed. Harvest 1927.)

[N = not affected. S = slightly affected. M = moribund. D = apparently dead.]

Degree of development and method of treatment	Loss on drying at 104° C. %	Concen- tration in terms of part of plant gm./100 c.c.	N %	S %	M %	D %	M & D %
Buds—closed.	16.8	0.35 and 0.2	—	—	—	100	100
Air-dried at ordinary tem- peratures		0.1	—	—	20	80	100
		0.05	20	10	50	10	60
		0.025	40	10	20	30	50
Buds beginning to show white petals.	16.8	0.35 and 0.2	—	—	—	100	100
Air-dried at ordinary tem- peratures		0.1	—	—	20	80	100
		0.05	10	30	40	20	60
		0.025	60	20	10	10	20
Flowers—half-open.	15.1	0.35 and 0.2	—	—	—	100	100
Air-dried at ordinary tem- peratures		0.1	—	—	50	50	100
		0.05	30	20	50	—	50
		0.025	80	10	—	10	10
Flowers—fully open.	14.5	0.35	—	—	—	100	100
Air-dried at ordinary tem- peratures		0.2	—	—	20	80	100
		0.1	—	—	40	60	100
		0.05	—	40	20	40	60
		0.025	50	20	20	10	30
Flowers—very fully open.	14.4	0.35 and 0.2	—	—	—	100	100
Air-dried at ordinary tem- peratures		0.1	—	—	—	100	100
		0.05	40	20	20	20	40
		0.025	30	20	50	—	50 (M)
Flowers—very fully open.	14.4	0.35	—	—	—	100	100
Dried at 45–50° C.		0.2	—	—	20	80	100
		0.1	—	—	—	100	100
		0.05	30	20	30	20	50
		0.025	60	30	10	10	20
Flowers—very fully open.	—	0.35	—	—	—	100	100
Soaked without drying in 95 % alcohol		0.2	—	—	70	30	100
		0.1	—	10	60	30	90
		0.05	50	30	20	—	20
Flowers—over-blown. Seeds not ripe.	14.8	0.35 and 0.2	—	—	—	100	100
Dried at 45–50° C.		0.1	—	10	10	80	90
		0.05	10	20	—	70	70
		0.025	50	20	—	30	30

*Effect of different methods of drying and after-treatment  
on the toxicity of the flowers.*

In a wet summer there might be some difficulty in drying a crop of pyrethrum in the ordinary way, and a few tests were made to ascertain whether artificial drying could be adopted without loss of toxic properties. Abbott's work<sup>(1)</sup> indicates that pyrethrum flowers can be subjected to dry heat at 120° C. for 18 hours without any noticeable injury, but temperatures of 130–140° C. for the same length of time destroy the toxic principle. Such high temperatures are not necessary for commercial drying, and these results indicate that there is a considerable margin of safety.

A large sample of fully open flowers was taken on July 19th, 1927, thoroughly mixed and divided into three equal portions which were treated in the following ways:

- (1) Dried in a drying room at 40–50° C. for 24 hours and ground before extraction.
- (2) Dried at ordinary temperatures, for 14 days and ground before extraction.
- (3) Put into 95 per cent. alcohol without drying or grinding.

There was a loss on drying in the first two cases of 72·5–73·3 per cent., the dried samples on heating in an electric oven to 104° C. showing a further loss of 14·4 per cent. on the partially desiccated samples. Sample (3) was filtered through muslin and freed by pressure as far as possible from the extract. The filtrate was then diluted to give a concentration of 10 per cent. calculated on the air-dried sample. Dilutions were prepared from this concentrated extract and their toxicities compared with extracts of similar concentrations prepared from samples (1) and (2). The results of this experiment, which are included in Table III, show that there is little or no difference between the three samples and that artificial drying or even direct pickling in alcohol can be employed after harvesting the crop without any material loss of toxic properties. The slightly lower toxicity values for the wet extracted sample (3) may be significant, but are certainly not of practical importance. They are probably due to incomplete extraction of the unground flower-heads and to the difficulty of freeing them entirely from the last traces of extract. On a large scale this difficulty would be readily overcome. It has, however, yet to be ascertained whether an extract containing so large an amount of water would retain its toxicity over a considerable period of time. It can be deduced from this experiment that difficulty

in air-drying need not be an insuperable objection to the growth and use of pyrethrum in this country.

*Toxicity of different parts of the plant.*

The results so far discussed have been in all cases concerned with the toxicity of the complete flower-heads, cut off from the stalk just below the receptacle. Comparative tests were also carried out on the stalks, and on the disc and ray corollae separately. These were air-dried in the usual way, and extracted with absolute alcohol. The values obtained are expressed in Table IV. It will be seen that the complete flowers were more than ten times as toxic as the stalks weight for weight; that the ray corollae were not toxic, but that the disc corollae were apparently rather more toxic than the stalks. The corollae are frequently shed in the drying process, but they are obviously of little commercial importance. Large quantities of stalks are however available, and if it is borne in mind that their toxicity is much less than that of the flowers, their use has some justification, provided they are treated by some suitable extraction process.

Table IV.

*Toxicities of different parts of pyrethrum plant to A. ruficollis.*

(Grown at Harpenden from Swiss seed. Harvest 1926.)

[N = not affected. S = slightly affected. M = moribund. D = apparently dead.]

Part of plant	Loss on drying at 104° C. %	Concentration in terms of part of plant gm./100 c.c.	N	S	M	D	M & D
			%	%	%	%	%
A. Flowers	14.8	0.5 and 0.35	—	—	—	100	100
Tests made Aug. 1926		0.2	—	20	40	40	80
		0.1	30	20	30	20	50
B. Flowers. Same extract as in A.		0.35 and 0.2	—	—	—	100	100
Tests made June 1927		0.1	10	—	—	90	90
		0.05	50	20	10	20	30
C. Flowers		0.5 and 0.35	—	—	—	100	100
Average of tests June–Aug. 1927		0.25 and 0.2	—	—	15	85	100
		0.1	7.5	20	25	47.5	72.5
		0.05	60	17.5	10	12.5	22.5
		0.025	80	10	—	10	10
D. Stalks	12.4	1.0	Not completely toxic				
Tested Aug. 1926							
E. Stalks		5.0	—	—	80	20	100
Tests made June 1927		2.5	—	—	30	70	100
		1.0	40	30	20	10	30
F. Disc corollae	15.1	1.0	—	20	20	60	80
G. Ray corollae	16.0	1.0	70	20	—	10	10

*Effect of exposure on the toxicity of pyrethrum.*

The data set out in Tables II and III were obtained some ten months after the crop had been harvested; the material had been stored in bulk in covered but not air-tight drums and tins, and the samples taken for testing had been stored after grinding in glass-stoppered bottles. It is commonly stated that pyrethrum powder loses its toxicity very readily, and although there is evidence in the literature against this opinion, it seemed advisable to make further experiments on the point.

Abbott<sup>(1)</sup> has shown that whole and ground flower-heads of pyrethrum are not injured if kept in sealed fruit-jars for 150 weeks; that ground flower-heads in closed glass vessels had lost their toxicity in  $5\frac{1}{2}$  years; but that whole flower-heads, kept under similar conditions were practically unhurt, even by such prolonged storage. Further, he found that whole and ground flower-heads could be exposed to the weather in an open dish for 12 weeks, without loss of toxicity, but that an exposure of 21 weeks reduced their potency; on the other hand, whole flower-heads exposed in an open dish in a room, retained their toxicity for 150 weeks, and ground flower-heads for 34 weeks, but the insecticidal value of the latter was reduced by an exposure to similar conditions in 136 weeks. It would appear from these results that pyrethrum under reasonable storage is very much more stable than is commonly supposed; our data tend to substantiate these conclusions.

Certain preliminary figures have been obtained indicating the effect of time on the toxicity of the flowers and stalks kept in stoppered bottles and upon the toxicity of the alcoholic extracts of the flowers. A sample of flowers grown from Swiss seed at Harpenden was harvested in July 1926 and tested in August of that year; the results are given under A (Table IV). Fresh extracts were made on several occasions from June to August in 1927, and the average figures are set out under C. The figures indicate no loss of toxicity on standing, the small differences that appear being almost certainly due to the difficulty of distinguishing with accuracy between the "moribund" and "dead" insects. In 1927 an opportunity occurred of testing the same alcoholic extract as was tested in August 1926, and the figures are set out under B. The difference between the values given under A and B are slight and within the limits of experimental error for this material, and the conclusion may be safely drawn that, between August 1926 and June 1927, the alcoholic extract had shown no loss of toxicity. In order to test the losses of toxicity under varying conditions of exposure a number of experiments

was made during the early part of 1927. A sample of coarsely ground flowers grown from Swiss seed in Harpenden and a sample of stalks grown from Swiss seed were exposed in shallow Petri dishes at Rothamsted in the insectary, a building which, while it afforded protection from

Table V.

*Effect of exposure on toxicity of pyrethrum to A. rumicis.*

(Grown at Harpenden from Swiss seed.)

[N = not affected. S = slightly affected. M = moribund. D = apparently dead.]

Treatment and length of exposure	Loss on drying at 104° C. %	Concentration in terms of part of plant gm./100 c.c.	N %	S %	M %	D %	M & D %
<i>First Series</i>							
Flowers—bottled sample	14.8	1.0-0.5	—	—	—	100	100
		0.25	—	—	30	70	100
		0.1	—	40	20	40	60
		0.05	40	40	—	20	20
Flowers—exposed 6 months in shallow dish to open air	14.3	2.0	—	—	—	100	100
		1.0	10	—	20	70	90
		0.5	—	20	10	70	80
		0.25	50	10	30	10	40
Flowers—moistened and exposed 13 days to air saturated with moisture. Much fungus infection	13.5	2.0-1.0	—	—	—	100	100
		0.5	—	10	20	70	90
		0.25	20	20	30	30	60
Flowers—moistened and exposed 2 months to air saturated with moisture (formalin present). No fungus	15.7	2.0-1.0	—	—	—	100	100
		0.5	—	40	20	20	40
		0.25	50	10	10	30	40
Stalks—bottled sample	12.4	5.0	—	—	40	60	100
		2.5	—	30	30	40	70
		1.0	40	10	20	30	50
		0.5	40	10	20	30	50
Stalks—exposed 6 months in shallow dish to open air	13.5	5.0	—	20	80	—	80
		2.5	60	20	20	—	20
<i>Second Series</i>							
Flowers—bottled sample	14.8	0.35	—	—	—	100	100
		0.2	—	—	20	80	100
		0.1	—	30	30	40	70
		0.05	60	30	10	—	10
Flowers—moistened and dried in shade	19.1	0.25	—	—	—	100	100
		0.1	50	—	—	50	50
Flowers—moistened and dried in sun	10.9	0.25	—	—	10	90	100
		0.1	60	10	30	—	30
Flowers—moistened and dried at 40-50° C.	11.4	0.25	—	—	—	100	100
		0.1	10	—	10	80	90
Flowers—kept moist 6 weeks in shade. Much fungus	11.7	0.5	50	20	10	20	30
		0.25	40	10	40	10	50(?)
		0.1	70	10	10	10	20
Flowers—kept moist in open, exposed to sun	12.9	0.5	—	—	30	70	100
		0.25	—	30	30	50	80
		0.1	60	40	—	—	—

rain and snow, could otherwise be regarded as giving conditions equivalent to the open air. The exposure continued for six months from December 8th, 1926 to June 8th, 1927, and the samples were tested on June 9th. The toxicity data and the moisture contents of the samples are set out in Table V together with the values given on this day by the samples stored in stoppered bottles.

The results indicate some loss of toxicity under these drastic conditions of exposure, though this was less than was expected. A sample of Japanese flowers exposed in the same way but not tested so critically showed little or no loss of toxic properties. The sample of stalks exposed in the same way for the same length of time gave values indicating a loss of toxicity.

Another sample of ground Swiss flowers was moistened and exposed in a shallow dish in a glass vessel, used normally as a desiccator, the atmosphere being kept saturated with water vapour. Within a fortnight a considerable growth of fungi was observed and the flowers were therefore air-dried and bottled. This sample showed a loss of toxicity. Another moistened sample was put into the apparatus, but in this case, in order to prevent fungal infection, a little formaldehyde was added to the water standing in the desiccator; after an exposure of two months, the powder was air-dried and tested; it showed some loss of toxicity. Powdered samples were also thoroughly wetted, and then dried in the sun, in the shade, and artificially dried; the loss of toxic properties observed was not great in any of the cases. Two further drastic tests were therefore made; samples in flat dishes were saturated with moisture, and exposed, one in the shade in the insectary, and the other on the roof of the laboratory. The latter sample frequently became flooded and on such occasions was allowed to dry indoors. The sample exposed in the insectary was kept thoroughly moist. The exposure continued for six weeks, by which time a plentiful growth of fungus had taken place on both samples. They were then air-dried and tested; both samples showed loss of toxicity, which in the case of the sample exposed in the insectary was considerable. Under sufficiently drastic conditions, therefore, pyrethrum can be made to lose its toxicity, but it is clear that the commonly expressed objection to its use on the grounds of a supposed rapid loss of toxicity is exaggerated. Obviously, after the crop is gathered, it should be dried at once to an extent which will not allow of fungus growth, but if then stored in a reasonable manner, *e.g.* in well-covered bins, there is no reason to expect any material loss of toxicity for a considerable period of time.

These conclusions are confirmed by some experiments with larvae of the purple thorn moth, the results of which are given in Table VI.

Table VI.

*Effect of exposure upon the toxicity of pyrethrum to S. tetralunaria (larvae).*

(Grown at Harpenden from Swiss seed.)

[N = not affected. S = slightly affected. M = moribund. D = apparently dead.]  
(Larvae 25-30 days old.)

Description	Concentration in terms of part of plant gm./100 c.c.	N	S*	M	D
Flowers—kept bottled	2.0	—	2	1	7
	1.0	—	2	1	7
	0.5	—	3	1	7
Flowers—exposed in insectary 8. xii. 26-8. vi. 27	2.0	—	2	1	7
	1.0	—	3	1	7
	0.5	—	3	—	7
Flowers—moistened and ex- posed to air saturated with moisture. Formalin present	2.0	—	—	3	7
	1.0	—	2	1	7
	0.5	—	1	—	9

\* Those recorded under S fed very little and made little or no growth but remained alive.

### *Toxicity of dried extracts of pyrethrum.*

In Tables I-VI concentrations are expressed as percentages of the air-dry flowers or stalks and the results indicate that the actual poison, which is present only to the extent of 0.4-0.5 per cent. of the flower-heads, must be extremely toxic. McDonnell, Roark and Keenan have shown that petroleum ether, whilst giving a smaller total weight of extracted matter than the other solvents, does extract the whole of the poison. Our experiments substantiate this finding. Five gm. of coarsely ground pyrethrum flowers were extracted in a Soxhlet apparatus successively with petroleum ether (B.P. 40-50° C.) ordinary methylated ether (Sp. Gr. .720) and absolute alcohol; after the extraction was completed, the solvent was evaporated on the water-bath to a small bulk and then to dryness *in vacuo*. The extracts were weighed, dissolved in absolute alcohol, diluted and tested for their insecticidal values. The petroleum ether extract was completely toxic down to a concentration of 0.01 per cent.

The extractions were done in duplicate, one being tested 14 and 71 days after extracting, having been allowed to stand in the meantime in the form of a concentrated emulsion. The second extract was allowed to stand 85 days in a dry state before testing. Both the petroleum ether extracts which had been allowed to stand for some time were found less

toxic than the sample tested shortly after extraction, indicating some loss of toxicity with time. Neither the ether extract after petroleum ether nor the alcoholic extract after ether showed appreciable toxicities.

#### EXPERIMENTS WITH CATERPILLARS.

In addition to the experiments with aphides, a limited number of tests were carried out with several species of lepidopterous larvae, the main object being to compare the degree of resistance to pyrethrum shown by different species. Extracts of flowers grown in Harpenden from Swiss seed were used. The technique adopted was similar to that used for aphides; each batch of larvae was sprayed in the standard apparatus and then transferred to separate small cages provided with a supply of fresh food-plant and kept under observation for 8–10 days. The numbers of larvae of two of the species available were rather small, but the results obtained were very consistent and definite and seemed worth putting on record.

Table VII.

*Toxicity of pyrethrum flowers to caterpillars of different species.*

(Grown at Harpenden from Swiss seed. Harvest 1926.)

[N=not affected. S=slightly affected. M=moribund. D=apparently dead.]

Species and age	Concentration in terms of part of plant gm./100 c.c.	N	S	M	D
<i>S. tetralunaria</i> (Purple thorn moth), 10–15 days old	2.0	—	—	—	10
	1.0	—	2	—	8
	0.5	—	2	—	7
	0.25	—	2	—	8
<i>S. tetralunaria</i> (Purple thorn moth), 25–30 days old	2.0	—	2	1	7
	1.0	—	2	1	7
	0.5	—	3	—	7
* <i>O. antiqua</i> (Vapourer moth), 3–4 weeks old	1.0	—	—	—	10
	0.5	—	—	—	10
	0.25	—	—	—	10
<i>M. brassicae</i> (Cabbage moth), 10–12 days old	0.5	10	—	—	—
<i>M. brassicae</i> (Cabbage moth), about 15 days old	2.0	7	—	—	3
	1.0	8	—	—	2
	0.5	10	—	—	—
	0.25	10	—	—	—
<i>Pieris brassicae</i> (Cabbage white butterfly), about half-grown	2.0	—	—	—	6
	1.0	—	—	—	6
	0.5	1	3	—	2
	0.25	2	2	—	2

\* Similar results were obtained with samples of pyrethrum from Newton Abbot, Isles of Scilly, and from France (p. 431).

Four species were tested—the purple thorn moth (*Selenia tetralunaria* Hufn.), the vapourer moth (*Orgyia antiqua* L.), the cabbage moth (*Barathra (Mamestra) brassicae* L.), and the large white cabbage butterfly (*Pieris brassicae* L.). The two former were bred from the egg under uniform and protected conditions and were completely free from parasites; the others were wild larvae, collected when quite small. In all cases, the larvae used in the experiments were in a comparatively young stage. The essential details and the results of the tests are given in Table VII.

It will be seen at once that the four species show very different powers of resistance to the toxic action of pyrethrum extracts.

The vapourer moth larvae proved to be very susceptible and all were killed by the spray at all concentrations down to 0.25 per cent.; the toxic action was extremely rapid.

The thorn moth larvae, which were tested at two stages of growth, were a little less easily killed; a small percentage survived at most concentrations, but these were in a semi-paralysed condition, and, though able to feed a little, made little growth.

The white cabbage butterfly larvae were rather older than the other species tested (about half-grown) and proved somewhat more resistant than the vapourer and thorn moths. All were killed however at concentrations of 2 and 1 per cent. At lower concentrations, four out of six survived, though some of these were obviously affected by the poison.

Finally, the cabbage moth larvae, even in a very young stage, showed themselves highly resistant, only two or three out of ten being killed at concentrations of 2 and 1 per cent. Lower concentrations had apparently no effect whatever, the treated larvae feeding and growing normally.

Such marked differences in the susceptibility of different species of insects to the action of pyrethrum have frequently been observed. Juillet, in chapters 14 and 19 of his monograph, discusses the toxic action of pyrethrum, and notes that, although most insects are susceptible, yet there are certain species which possess high powers of resistance, and includes among these *Barathra (Mamestra) brassicae*, the cabbage moth.

A single experiment with cabbage moth larvae was made to ascertain whether pyrethrum extract would have an action as a stomach poison. Young cabbage plants in pots were sprayed with fluids containing 1 per cent. and 0.5 per cent. of the flowers with 0.25 per cent. soft soap, and after being allowed to dry, young larvae were caged on the plants. The larvae were apparently entirely unaffected; they fed on the sprayed foliage, grew normally, and were indistinguishable from the control larvae

feeding on plants sprayed with 0.25 per cent. of soft soap only, or unsprayed. There was no evidence that the pyrethrum extract had any action as a stomach poison or as a repellent to these insects.

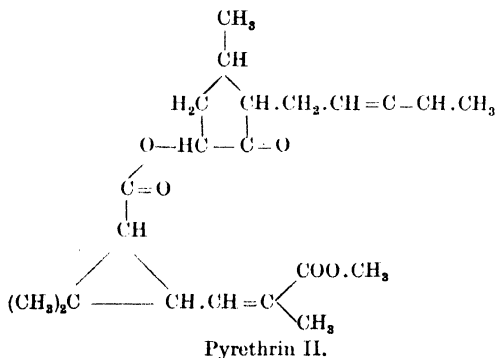
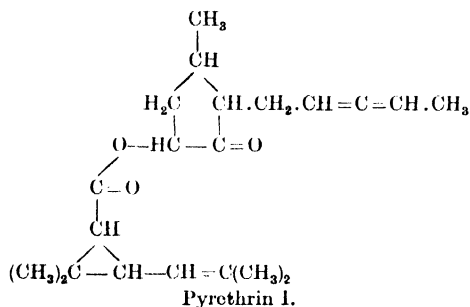
#### DISCUSSION.

The data given in the experimental part of this paper confirm the results of other workers as to the high insecticidal value of pyrethrum (*C. cinerariaefolium*), and show that this plant can be successfully grown in England and harvested without loss of toxicity under our climatic conditions. The material raised here has about the same insecticidal value as samples received from abroad. The results also indicate that the crop can be air-dried at ordinary temperatures, or artificially dried if necessary, without damage, and that the air-dried flowers may be stored in covered vessels for considerable periods with little or no deterioration. Prolonged exposure to wet conditions may however lead to some loss of toxicity. We find no justification, so far as the toxicity of the product is concerned, for the opinion that it is better to harvest the crop when the flowers are "half-closed" than when they are fully open. We have not however dealt with the bearing of this practice on the detection of subsequent adulteration of the dried flowers.

McDonnell, Roark and Keenan (6) deal with this phase of the pyrethrum question at some length, and they have suggested microscopical and chemical methods for the detection of adulteration. Their chemical methods do not, however, depend upon the isolation or determination of any constituent which is directly colligative with the toxic principle of the plant. This indeed was impossible until the active principle was discovered and its constitution determined. A satisfactory method of comparing the toxicities of different samples of pyrethrum either directly by biological methods or by estimation of the toxic constituents would be of considerable economic importance for the selection of varieties with higher poison contents and for the evaluation of individual samples.

The biological method used in the work described in this paper has proved admirably adapted for the purpose of evaluating the samples we have had to test, and has indeed been used for the detection of pyrethrum extracts in insecticidal material of unknown composition. The detection of major differences in toxicity between different samples has been aimed at in the present work, but, by using larger numbers of insects and concentrations nearer together, smaller differences in toxicity could undoubtedly be detected and estimated by this method.

In recent years, a considerable amount of research has been carried out on the chemical composition of the active principles of pyrethrum. In 1909, Fujitani(4) isolated a thick yellow syrup, highly toxic to insects, to which the name Pyrethron was given, and this material was further studied by Yamamoto(12); we are however chiefly indebted to the classical researches of Staudinger and Ruzicka(9) for our knowledge of the constitution of the active principles. These investigators isolated in a pure state two chemical compounds highly poisonous to insects, named by them Pyrethrin I and II, and the structure of both compounds was elucidated. The constitutional formulae ascribed to them are as follows:



Both of these bodies are excessively toxic to insects. Pyrethrin I is the more active and, according to these authors, killed cockroaches at a dilution of 1 in 10,000 in 10 to 20 min.; pyrethrin II was less toxic and required 20 to 40 min.

Staudinger and Ruzicka ascribe the high toxicity of the pyrethrins to their peculiar structure as esters of trimethylene carboxylic acids with unsaturated side chains and a cyclopentalone derivative with an unsaturated side chain. Small changes in either the alcoholic or acidic portions of the molecule caused a profound reduction in toxic properties. They found between 0.2 and 0.3 per cent. of pyrethrin in the flower-heads

of the samples they were dealing with. The isolation of these compounds in a pure state is complicated and depends on the preparation of their semi-carbazones.

It is evident from the work of these authors that any chemical method for the quantitative evaluation of pyrethrum which aims at a separation of the semi-carbazones of the actual insecticidal substances present is likely to be difficult and unsuitable for routine work. They themselves suggest that pyrethrin might be determined by isolating the semi-carbazones of the alcohol pyrethrolone but the process is not a simple one.

Recently, Staudinger and Harder(s) have published an account of two somewhat simpler methods of estimating pyrethrin I and II, and found that the pyrethrin content of a number of samples of pyrethrum flowers ranged from 0.4 to 0.6 per cent., a higher figure than that obtained by the older method. The pyrethrin content of the stalks varied between 0.04 and 0.1 per cent. The data at present available indicate that the difference in the pyrethrin content of flowers and stalks is of the same order as the difference in the toxicity of flowers and stalks as determined by our biological method. Further, Staudinger and Harder found only insignificant differences between the pyrethrin contents of closed, half-open and fully open flowers, which is again in agreement with the results of our toxicity experiments. We hope, at a later date, to discuss the chemical method of Staudinger and Harder in greater detail and to compare the results further with those obtainable by the biological method.

It would be of interest to ascertain the pyrethrin content of samples of genuine pyrethrum in which toxicity had been lost, as *e.g.* by prolonged exposure to wet conditions. The most probable explanation of such loss of toxicity is that it is due to hydrolysis of the Pyrethrin. The losses of toxicity in alcoholic-soap extracts of pyrethrum (*Savon-pyrèthre*) are thus explained by Staudinger and Harder. Another source of loss, however, pointed out by these investigators, is one due to alcohol-radical exchanges (ester exchange); methyl alcohol gives rise readily to the methyl esters of the acids and free pyrethrolone which are not toxic, and though ethyl alcohol apparently reacts more slowly, it is nevertheless not considered by them to be suitable for technical extracts and they propose for the purpose indifferent organic solvents such as benzene, petroleum ether, trichlorethylene and acetone. Harder has shown that extracts of this type can be emulsified by turkey-red oil and are quite suitable for use in insect control. So far, in our experiments, extracts of pyrethrum containing 14 to 15 per cent. of moisture made with absolute

ethyl alcohol have retained their toxicity for many months; we have not, however, determined the length of time that the toxicities of extracts made with commercial spirit would be maintained.

# SUMMARY.

1. The toxicity to *Aphis rumicis* L. and to certain caterpillars of spray fluids prepared from samples of pyrethrum (*Chrysanthemum cinerariaefolium*) grown in England from Swiss and Japanese seed have been quantitatively determined.

2. Pyrethrum flowers, grown in six different localities, showed only slight differences, and, for practical purposes, all the samples had approximately the same toxicity. They did not differ in this respect significantly from a sample grown on the continent.

3. The toxicities of extracts of equal weights of pyrethrum flowers tested at different stages of development differed very little.

4. Artificial drying of the flowers had no significant effect on the toxic properties.

5. The flowers were about ten times as toxic as the stalks, weight for weight.

6. Prolonged exposure of pyrethrum to wet conditions led to some loss of toxicity, but contrary to the usual opinion, if stored in a reasonable manner, it remained for long periods without deterioration.

7. Caterpillars of different species showed marked differences in susceptibility to the action of pyrethrum.

8. The biological method employed has proved suitable for evaluating samples of pyrethrum.

# LITERATURE.

- (1) ABBOTT, W. S. (1919). *U.S. Dept. Agric. Bull.* 771.
- (2) FAES, H. (1918). *Journ. suisse Pharm.* LVI, 429.
- (3) FRYER, J. C. F. and STENTON, R. (1927). *Journ. Min. Agric.* XXXIII, 916.
- (4) FUJITANI, J. (1909). *Arch. exp. Path. u. Pharm.* LXI, 47.
- (5) JULLET, A. (1924). *Le Pyrèthre Insecticide*. Rommégous et Déhan, Montpellier.
- (6) McDONNELL, C. C., ROARK, R. C. and KEENAN, G. L. (1920). *U.S. Dept. Agric. Bull.* 824.
- (7) PASSERINI, N. (1919, 1920). *Nuovo Giorn. bot. ital.* XXVI, 30; XXVII, 1.
- (8) STAUDINGER, H. and HARDER, H. (1927). *Ann. Acad. Scient. Fennicae*, A, XXIX, No. 18.
- (9) STAUDINGER, H. and RUZICKA, L. (1924). *Helv. Chim. Act.* VII, 177.
- (10) TATTERSFIELD, F., GIMINGHAM, C. T. and MORRIS, H. M. (1925). *Ann. App. Biol.* XII, 61. (*Rothamsted Memoirs*, XII.)
- (11) TATTERSFIELD, F. and MORRIS, H. M. (1924). *Bull. Ent. Res.* XIV, 223. (*Rothamsted Memoirs*, XII.)
- (12) YAMOMOTO, R. (1925). *Inst. Phys. Chem. Res. Tokyo*, III, 193.

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## LABORATORY EXPERIMENTS WITH NON-ARSENICAL INSECTICIDES FOR BITING INSECTS

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(With Plate XXXVII.)

THE use of fluids or dusts containing arsenic compounds for the control of biting insects of economic importance is attended by certain obvious disadvantages and a good deal of work has been done in recent years in the attempt to find satisfactory substitutes for "arsenicals." In an interesting paper<sup>(1)</sup> Moore and Campbell give a brief account of preliminary laboratory experiments with a considerable number of compounds, both organic and inorganic, as stomach poisons. The work had specially in view the control of the Japanese beetle (*Popillia japonica*), but other beetles and Tent caterpillars were also used. The insects, usually 20 in number, were placed in a cage, and sprayed or dusted foliage of either potted plants or freshly cut shoots in water was then introduced. The number of insects dead at the end of each 24-hour period was recorded, together with observations on the amount of feeding and on injury to the foliage, if any. The results were chiefly of theoretical interest, copper cyanide being the only non-arsenical tested which had a toxicity to the Japanese beetle comparable with that of lead arsenate. It caused no damage to foliage. Copper thiocyanate showed high toxicity to Tent caterpillars but was non-toxic to Japanese beetle. Hargreaves<sup>(2)</sup> has also tested a number of miscellaneous organic compounds. Emulsions of the various substances with a liquid soap were prepared, and after immersing leaves in 90 per cent. alcohol and allowing them to become nearly dry, the mixtures were applied with a brush. Larvae of *Pieris rapae* on cabbage and *Spilosoma lubricipeda* on lupins (5 larvae per test) were used, but many of the experiments were complicated by the fact that some of the larvae were parasitised. Salts of the dinitro-cresols, some naphthalene derivatives and barium and calcium fluorides are

recorded as having high toxicity. No reference is made to the effect of the chemicals on the foliage. The dinitro-cresylates are known to be highly injurious to many kinds of foliage<sup>(3)</sup> and therefore, although very toxic, they are not likely to be of practical interest as possible ingredients of spray-fluids for summer use.

Among possible substitutes for arsenicals, perhaps most attention has been paid to compounds containing fluorine. The toxicity of sodium fluoride to cockroaches was investigated by Shafer<sup>(4)</sup> and it is frequently used in poison baits for these insects; more recently its possibilities as a locust poison have been pointed out by Ripley<sup>(5)</sup>, who found that baits of grass or fresh horse dung moistened with a 2 per cent. solution of sodium fluoride or dusted with a 1 : 50 mixture of sodium fluoride and lime proved highly effective. It is less poisonous but also less repellent<sup>1</sup> to the insects than sodium arsenate. Unfortunately, sodium fluoride solutions, even when very dilute, cause serious damage to foliage and are therefore not suitable for use against leaf-eating insects in the form of summer spray-fluids. This objection is not shared to the same extent by certain silicofluorides. These compounds have long been known to have insecticidal and fungicidal properties, and Marcovitch has recently published a number of papers<sup>(6)</sup> on the efficiency of sodium and calcium silicofluorides, when used in the form of dusts, as insecticides for biting insects. Sodium silicofluoride mixed with 9 parts of hydrated lime gave good control against the Mexican bean beetle (*Epilachna corrupta*) and other insects and can also be used alone, at all events on certain plants. Calcium silicofluoride is similarly effective, but when undiluted causes severe foliage injury; it can however apparently be safely used mixed with lime or other diluents, and dusting preparations of this material are now on the market.

Roark<sup>(7)</sup> has pointed out that commercial grades of sodium silicofluoride may contain some sodium carbonate, and that in the presence of water the silicofluoride would be decomposed by the alkali and sodium fluoride formed. This would cause injury to foliage. Similarly, if mixed with hydrated lime, sodium silicofluoride would be converted into calcium silicofluoride and then into calcium fluoride. Alkaline water or alkaline exudations from the plants to which the material is applied might also bring about formation of fluorides. Roark suggests that the formation of fluorides accounts, at all events to some extent, for the insecticidal action of silicofluorides and that the use of some of the less

<sup>1</sup> H. W. Thompson has, however, found that a sodium fluoride poison bait is of little use against "leatherjackets." (*Welsh Journ. Agr.* 1926, II, 228.)

soluble fluorides which are not injurious to foliage would result in more uniform insecticidal action. Marcovitch however maintains that the silicofluorides are more effective than relatively insoluble fluorides and that they can be safely used.

More recently a report has appeared from the United States Chemical Warfare Service Cotton Boll Weevil Investigation, by Walker and Mills<sup>(8)</sup>, which gives an account of laboratory and field experiments with a large number of substances tested as dusts against the cotton boll weevil (*Anthonomus grandis*). Ordinary commercial sodium silicofluoride was found more toxic than commercial calcium arsenate volume for volume, but owing to its greater density two to four times the weight was required to cover the same area. A special form of sodium silicofluoride was however prepared, having a much lower density, which was found to be as effective weight for weight as calcium arsenate and which caused only negligible injury to cotton. A specially prepared barium silicofluoride was similarly effective. Both compounds were superior to calcium arsenate in adhesive power. Further field experiments are planned.

Langford<sup>(9)</sup> and other workers in America have successfully used sodium silicofluoride as a poison in baits for grasshoppers and other insects.

Quantitative data in regard to the efficiency of stomach poisons are not easy to obtain. Some interesting work in this direction has been done recently by Campbell<sup>(10)</sup> who has based a quantitative method on the observation that certain caterpillars will absorb completely drops of liquid placed on the leaf in their feeding path, if the liquid is not too distasteful. By suitable manipulation individual larvae can thus be given known doses of toxic substances, and the times of survival noted. A quantitative basis for "comparison of susceptibilities" can thus be arrived at. The silkworm is considered to be specially suitable for this type of work. The results so far reported deal with arsenic compounds, and it has been shown by this method that the susceptibility of silkworms (*i.e.* the effects of equal doses per unit weight of animal) to arsenic decreases during larval development. Campbell has also used his technique to investigate the interesting question of the possible development of tolerance to arsenic, and preliminary experiments show that individual tolerance to arsenic was not induced in silkworms by quantitative feeding of sublethal doses of sodium arsenate solution.

Janisch<sup>(11)</sup> has described a method which aims at obtaining quantitative data by weighing the poisoned leaves and tracing their outlines

on squared paper before and after the insects have fed on them and thus ascertaining the quantity of poison ingested.

Van Leeuwen<sup>(12)</sup>, working on the toxicity of lead arsenate to the Japanese beetle (*Popillia japonica*), measured the area of treated foliage consumed and the quantity of arsenic causing death; and Newcomer<sup>(13)</sup> has described a technique suitable for use in laboratory experiments on the toxicity of stomach poisons to Codling moth larvae.

A detailed account has also been published by Kalandadze<sup>(14)</sup> of experiments with arsenical dusts on several insects which are pests of forest trees. Large glass cylindrical jars were used as cages with the foliage in water in a small bottle with cotton-wool plug. In each series of experiments the same amount of foliage was used in each cage, leaves or shoots being counted, and the amount eaten at the end of the experiment determined, either as surface area or as number of leaves or needles. Larvae of *Lymantria dispar*, *L. monacha* and *Bupalus piniarius* were tested at each instar and were allowed to complete development if possible. The arsenic content of some of the poisoned insects was determined, and the excreta in each cage collected and weighed at the end of the experiment. Among other results it was found that the minimum toxic dose of arsenic increased with the age of the larvae and that freshly moulted individuals are more susceptible than those which have moulted some time. Partially poisoned larvae often recovered when given fresh foliage, but such recovery was sometimes only apparent, the larvae failing to complete development. In the case of larvae which received a sublethal dose of arsenic and did succeed in completing development, there appeared to be an "after-effect" of the poison since eggs laid by adults arising from such larvae almost always failed to hatch.

In the past few years the writers have made laboratory experiments on the toxicity of various substances as stomach poisons to biting insects, when applied to foliage as sprays. A simple technique is employed for these tests, and although it does not differ in principle from methods adopted by other workers a short description may be of interest.

Hurricane lamp glasses are used as the separate cages required for each test. They are fitted with bungs at the smaller end, each bung having a hole bored through the middle. Shoots of the food plant of convenient size are cut and the foliage stripped off at the lower end so as to give a long stalk. The foliage is sprayed, by means of a small bottle sprayer, with the liquids to be tested and the shoots suspended head downwards until dry; each is then placed in a lamp glass, the stalk being pushed

through the hole in the cork. The glasses are supported in any suitable manner with the stalks dipping into water; wooden trays with spaces between the slats are convenient, with a separate test-tube below for each stalk. With plants such as hawthorn, hazel, black currant, etc., the foliage keeps fresh for 10 days or longer, if the water is renewed as required. A counted number of caterpillars or other insects are placed in each cage and the top covered with muslin attached to an iron ring, which holds it in place and avoids the need for tying<sup>1</sup>. When very young larvae are used, a tight fit where the stalk of the shoot passes through the cork is ensured by packing with a little cotton wool. Fig. 1 shows a single cage containing a shoot of hawthorn, supported on a tripod, and Fig. 2 a dozen arranged on a tray. With cages of this type a considerable number of tests can be run concurrently without much trouble and at no great expense, and it is a simple matter to examine the insects at intervals, and to observe the extent of feeding and the effect of the spray-fluid on the foliage. At each examination the number of insects "unaffected," "slightly affected," "moribund" and "dead" are recorded; and in the case of larvae notes are also made in regard to their growth.

It is advisable to carry on experiments of this type for not less than 8 or 10 days before drawing definite conclusions. It has been observed by Krasilschtschik (15) that in many cases the maximum mortality is not reached until about the eighth day; and this has also been our experience. Except in cases where the substance used renders the foliage very repellent, larvae which survive beyond the eighth day are usually capable of completing their development. Several species of lepidopterous larvae, including *Selenia tetralunaria*, *Orgyia antiqua*, *Abraxas grossulariata*, and *Cheimatobia brumata* have been used, stocks being reared under as natural conditions as possible, but protected from the attacks of parasites.

It is not proposed to discuss the results obtained in any detail because there are a number of irregularities and discrepancies which will need much further work before they can be cleared up. A brief reference to the kind of results obtained with two dissimilar groups of compounds may, however, serve a useful purpose.

The silicofluorides<sup>2</sup> are perhaps of special interest since, as already mentioned, they have been recommended for use as the toxic ingredient

<sup>1</sup> The writers are indebted to Mr E. E. Green, through Dr A. D. Imms, for this convenient means of covering small cages.

<sup>2</sup> A note on some early experiments with silicofluorides was published in *Ind. Eng. Chem.* 1925, xvii, 323.

Table I.  
*Silicofluorides.*

Substance	Concentration in gm. per 100 c.c.	Larvae	% not af- fected	% dead	Foliage	Injury to foliage	Date of Exp.
Sodium silicofluoride	1.0-0.5	<i>C. brumata</i>	—	100	Apple	Severe	25. v.
	0.25	"	—	80	"	"	"
	1.0 & 0.75	<i>S. tetralunaria</i> (young)	—	100	Hawthorn	Variable:	29. v.
	0.5 & 0.25	"	—	100	"	{ not great	"
	0.5	<i>S. tetralunaria</i> (older)	—	100	"	None	17. vi.
	0.25	"	—	40	"	Slight	"
	0.1	"	100	—	"	None	"
	0.5	<i>S. tetralunaria</i> (young)	—	100	"	Considerable	15. viii.
	0.25	"	—	80	"	Slight	"
	0.1	"	100	—	"	Traces	"
	0.5	<i>A. grossulariata</i>	—	40	Black currant	Some	17. viii.
	0.25	"	—	23	"	Traces	"
	0.1	"	100	—	"	None	"
Potassium silicofluoride	1.0-0.5	<i>S. tetralunaria</i> (young)	—	100	Hawthorn	None	29. v.
	0.25	"	—	80	"	"	"
	0.5	<i>S. tetralunaria</i> (older)	—	30	"	"	17. vi.
	0.25	"	30	10	"	"	"
	0.1	"	100	—	"	"	"
	0.5	<i>S. tetralunaria</i> (young)	—	80	"	Severe	15. viii.
	0.25	"	—	60	"	Slight	"
	0.5	<i>A. grossulariata</i>	—	60	Black currant	None	17. viii.
	0.25	"	—	40	"	"	"
	0.1	"	80	20	"	"	"
	1.0	<i>S. tetralunaria</i> (young)	—	100	Hawthorn	None	29. v.
	0.75	"	—	55	"	"	"
	0.5	"	—	40	"	"	"
Aluminium silicofluoride (B.D.H.)	0.25	"	50	30	"	"	"
	1.0	<i>S. tetralunaria</i> (older)	—	60	"	"	17. vi.
	0.75	"	—	70	"	"	"
	0.5	"	10	40	"	"	"
	0.25	"	100	—	"	"	"
	1.0	<i>S. tetralunaria</i> (young)	—	80	"	Slight	15. viii.
	0.5	"	—	40	"	Severe	"
	0.25	"	—	—	"	"	"
	1.0	<i>A. grossulariata</i>	80	—	Black currant	None	17. viii.
	0.5	"	80	—	"	Very slight	"
	0.25	"	80	—	"	None	"
	1.0-0.75	<i>S. tetralunaria</i> (older)	—	100	Hawthorn	Traces	17. vi.
	0.5	"	—	60	"	"	"
Aluminium* silicofluoride	0.25	"	—	50	"	Slight	"
	0.5	<i>S. tetralunaria</i> (young)	50	30	"	Severe	15. viii.
	0.25	"	30	20	"	"	"
	0.1	"	50	—	"	Considerable	"
	0.5	<i>A. grossulariata</i>	—	30	Black currant	"	17. viii.
	0.25	"	50	—	"	Very slight	"
	0.1	"	50	—	"	Traces	"
	1.0-0.75	<i>S. tetralunaria</i> (young)	—	100	Hawthorn	Irregular	29. v.
	0.5	"	—	100	"	Very slight	"
	0.25	"	50	50	"	"	"
	1.0-0.75	<i>S. tetralunaria</i> (older)	—	100	"	Considerable	17. vi.
	0.5	"	—	20	"	? traces	"
	0.25	"	—	40	"	Some	"
Calcium silicofluoride	1.0	<i>S. tetralunaria</i> (young)	100	—	"	Severe	15. viii.
	1.0	<i>A. grossulariata</i>	100	—	Black currant	Some	17. viii.
	1.0-0.5	<i>S. tetralunaria</i> (young)	—	100	Hawthorn	None	29. v.
		"	100	—	"	—	15. viii.
		<i>S. tetralunaria</i> (older)	95	5	"	—	17. vi.
Lead Arsenate		<i>A. grossulariata</i>	100	—	Black currant	—	17. viii.
		"	100	—	"	—	"
		"	100	—	"	—	"
Controls		"	100	—	"	—	"
		"	100	—	"	—	"
		"	100	—	"	—	"

\* Precipitated from aluminium sulphate using a slight excess of sodium silicofluoride.

of dusting preparations. Used as spray-fluids in solution or suspension in a 1 per cent. solution of saponin, we have found the silicofluorides of sodium, potassium, calcium and aluminium to have considerable toxicity as stomach poisons to young larvae of several species of moths; but the power of resistance varies with the different species and is markedly greater with older larvae. In regard to injury to foliage the results were extremely variable and difficult to interpret; the extent of injury not only differed with different plants but also with the same plant on different occasions. The observations indicate that, with hawthorn, the foliage is more sensitive to injury by silicofluorides in the latter part of the summer than in May or June.

Table I gives some examples of the kind of data obtained.

It is evident that, in spite of the considerable toxicity of various silicofluorides, they cannot be suggested at the present stage even for larger scale field experiments as sprays for use on foliage. There are some conditions under which these compounds appear to cause little or no damage to foliage, but a much more extensive series of laboratory experiments than it has been possible to make at present is required to establish these conditions.

Results of a quite different kind were given by the use of extracts of certain tropical leguminous plants known to have a high toxicity as contact insecticides(16). In this case the question of risk of injury to foliage does not arise; the extracts are quite harmless to plants. The outstanding fact shown by the experiments with these materials was their extremely repellent action. Foliage sprayed with extracts of these plants even at high dilutions remained untouched by the larvae in almost every case; rather than eat it, the larvae eventually died of starvation. Details of some tests with *Tephrosia vogelii*, *T. toxicaria* and *T. macropoda*, and with Black and White Haiari (species of *Lonchocarpus* from British Guiana) are given in Table II.

The possible practical value of repellents in combating leaf-eating insects has been little worked on; it might be considerable in special cases and would seem worth following up.

Results from the type of experiments discussed indicate a marked degree of specificity in the resistance of insects to stomach poisons and in the action of different substances upon foliage. They call for a more quantitative investigation of the whole subject.

F. L. Campbell(17) has recently put forward a plea for the development of laboratory research on the effects of poisons on insects on strictly quantitative lines, and his work on quantitative methods for the in-

Table II.

*Extracts of some tropical plants.*

(N=not affected: S=slightly affected: M=moribund: D=dead.)

Extract of	Concen- tration expressed as % of plant material	Larvae on hawthorn	N %	S %	M %	D %	Feeding
Tephrosia	2.0	<i>O. antiqua</i> (half grown)	—	90	—	10	Practically none: starved
Vogelii	1.0	"	—	90	—	10	" "
(leaves)	0.75	"	—	70	20	10	" "
	0.5	"	10	90	—	—	Very little: slight growth
Black	1.0	"	—	20	60	20	Practically none: starved
Haiari	0.75	"	—	80	20	—	Very little: starved
(stems)	0.5	"	—	80	20	—	" "
	0.25	"	10	50	40	—	Considerable
Black	1.0	"	—	70	30	—	Practically none: starved
Haiari	0.75	"	—	100	—	—	" "
(roots)	0.5	"	—	80	20	—	" "
	0.25	"	20	60	20	—	Considerable
White	1.0	"	20	80	—	—	Evident, but less than normal
Haiari	0.75	"	30	70	—	—	" "
(stems)	0.5	"	50	50	—	—	" "
	0.25	"	100	—	—	—	Normal
White	1.0	"	—	90	10	—	Very little: starved
Haiari	0.75	"	—	80	—	20	" "
(roots)	0.5	"	—	100	—	—	Little
	0.25	"	70	30	—	—	Considerable
Controls	—	"	100	—	—	—	Normal
	—	"	100	—	—	—	"
	—	"	100	—	—	—	"
Tephrosia	2.0	<i>S. tetralunaria</i> (young)	—	—	—	100	None: starved
Vogelii	1.0	"	—	—	—	100	"
(leaves)	0.5	"	—	—	20	80	"
	0.25	"	—	100	—	—	Evident, but less than normal
Black	2.0	"	—	—	—	100	None: starved
Haiari	1.0	"	—	—	—	100	"
(stems)	0.5	"	—	—	50	50	"
	0.25	"	—	90	—	10	Some: slight growth
Black	1.0	"	—	—	—	100	None: starved
Haiari	0.5	"	—	—	—	100	Very little: starved
(roots)	0.25	"	—	60	—	40	" "
White	1.0	"	—	—	—	100	None: starved
Haiari	0.5	"	—	—	—	100	"
(stems)	0.25	"	—	80	—	20	Very little: starved
White	1.0	"	—	—	—	100	None: starved
Haiari	0.5	"	—	—	—	100	"
(roots)	0.25	"	—	—	40	60	"
Controls	0.25 % soap	"	100	—	—	—	Normal: good growth
	"	"	100	—	—	—	" "
Unsprayed	"	"	100	—	—	—	" "

Table II (*continued*).

(N=not affected: S=slightly affected: M=moribund: D=dead.)

Extract of	Concentration expressed as % of plant material	Larvae on hawthorn	N %	S %	M %	D %	Feeding
White Haiari (stems)	1.0	<i>C. brumata</i> (young)	—	—	—	100	None*
	0.5	"	—	—	—	100	
White Haiari (roots)	1.0	"	—	—	—	100	
	0.5	"	—	—	—	100	
Black Haiari (stems)	1.0	"	—	—	—	100	
	0.5	"	—	—	—	100	
Black Haiari (roots)	1.0	"	—	—	—	100	
	0.5	"	—	—	—	100	
Tephrosia Vogelii (leaves)	1.0	"	—	—	—	100	
	0.5	"	—	—	—	100	
Tephrosia Toxicaria (roots)	1.0	"	—	—	—	100	
	0.5	"	—	—	—	100	
Tephrosia Macropoda (stems)	1.0	"	—	—	—	100	None*
	0.5	"	—	—	10	90	
Tephrosia Macropoda (roots)	1.0	"	—	—	—	100	None*
	0.5	"	—	—	—	100	
Controls	0.25 % soap	"	100	—	—	—	Normal: larvae pupated
	Unsprayed	"	100	—	—	—	" "
Black Haiari (stems)	1.0	<i>T. gothica</i> (10-14 days old)	—	100	—	—	Appreciable feeding but very little growth
	0.5	" "	—	100	—	—	Appreciable feeding and some growth, but less than normal
Controls	0.25 % soap	" "	100	—	—	—	Normal
	Unsprayed	" "	100	—	—	—	"

\* Results were identical on apple and hawthorn. Strong repellent action in all cases so that larvae died of starvation. 0.25 % soap was used with all the extracts.

vestigation of stomach poisons has already been referred to. We have long been convinced that the quantitative method of approach to the problems of insecticides and insecticidal action is the only one likely to lead to further advances of economic importance, and we have for some years been engaged on the study of contact insecticides from a quantitative point of view. Our experience in regard to work with stomach poisons is less extensive, but the results discussed serve to emphasize the need for a knowledge of insect toxicology (to use Campbell's term) on a systematic quantitative basis.

## SUMMARY.

1. A convenient technique for experiments with insecticides for biting insects is described.

2. The silicofluorides of sodium, potassium, aluminium and calcium, used in the form of spray-fluids, showed considerable toxicity to young larvae of several species of moths. The degree of resistance varies with different species and is greater with older larvae. Considerable, but irregular, injury to foliage was noted, and much further work is required to establish the conditions under which these compounds could be safely used.

3. Foliage sprayed with extracts of certain tropical plants is extremely repellent to young larvae. Even with high dilutions of the extracts, the foliage remained uneaten and the larvae eventually died of starvation.

4. A short review of some recent work on laboratory experiments with non-arsenical insecticides for biting insects is given.

## LITERATURE CITED.

- (1) MOORE, W. and CAMPBELL, F. L. (1924). *Journ. Agr. Res.* xxviii, 395.
- (2) HARGREAVES, E. (1924). *Bull. Ent. Res.* xv, 51.
- (3) TATTERSFIELD, F., GIMINGHAM, C. T. and MORRIS, H. M. (1925). *Ann. App. Biol.* xii, 218.
- (4) SHAFER, G. D. (1916). *Mich. Agr. Coll. Expt. Sta., Tech. Bull.* 21.
- (5) RIPLEY, L. B. (1924). *Bull. Ent. Res.* xv, 29.
- (6) MARCOVITCH, S. (1924). *Tenn. Agr. Expt. Sta. Bull.* 131; 1924, *Ind. Eng. Chem.* xvi, 1249; 1925, *Science*, lxi, 22; 1925, *Journ. Econ. Ent.* xviii, 122; 1926, *Tenn. Agr. Expt. Sta. Bull.* 134; 1926, *Journ. Econ. Ent.* xix, 795; 1926, *Science*, lxiv, 159.
- (7) ROARK, R. C. (1926). *Science*, lxiii, 431.
- (8) WALKER, H. W. and MILLS, J. E. (1927). *Ind. Eng. Chem.* xix, 703.
- (9) LANGFORD, G. S. (1926). *Journ. Econ. Ent.* xix, 670.
- (10) CAMPBELL, F. L. (1926). *Journ. Agr. Res.* xxxii, 359; *Journ. Econ. Ent.* 1926, xix, 516; *Journ. Gen. Physiol.* 1926, ix, 433, 727.
- (11) JANISCH, R. (1926). *Nachrichtenbl. deut. Pflanzenschutzdienst.* vi, 18.
- (12) LEEUWEN, E. R. VAN (1927). *Journ. Agr. Res.* xxxiv, 1043.
- (13) NEWCOMER, E. J. (1926). *Ibid.* xxxiii, 317.
- (14) KALANDADZE, L. (1927). *Zeit. Angew. Ent.* xiii, 1.
- (15) KRASILSCHTSCHIK, J. M. (1903). *Arb. Ent. Bureau, St Petersburg*, iv (Abstract in *Z. wiss. Insektenbiol.* 1910, vi, 120).  
 Cf. also BARSACQ, J. (1907). *Rev. Sci.* vii, 721.
- (16) TATTERSFIELD, F., GIMINGHAM, C. T. and MORRIS, H. M. (1925). *Ann. App. Biol.* xii, 61; 1926, xiii, 424.
- (17) CAMPBELL, F. L. (1926). *Science*, lxiii, 45.

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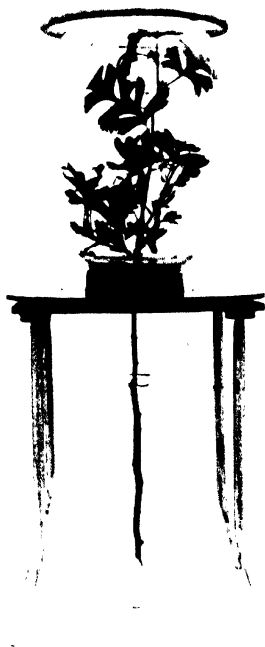


Fig. 1. Apparatus used for experiments with stomach poisons: a single cage.



Fig. 2. Apparatus used for experiments with stomach poisons.



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# PYRETHRIN I AND II.

## THEIR INSECTICIDAL VALUE AND ESTIMATION IN PYRETHRUM (*CHRYSANTHEMUM CINERARIAEFOLIUM*). I.

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(With Five Text-figures.)

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### INTRODUCTION.

CERTAIN varieties of Pyrethrum, chiefly those derived from Persia and the Near East, have been employed for at least a century as household insecticides. The use of one of these, *Chrysanthemum cinerariaefolium*, for household and horticultural purposes has spread from Dalmatia, where it appears to have been first extensively grown, to all parts of the world; its cultivation is now widely practised particularly in Japan, France and Switzerland. Its further cultivation in temperate

<sup>1</sup> F. T. and R. P. H. were responsible for the chemical, C. T. G. for the entomological side of this investigation. The spray trials were done conjointly.

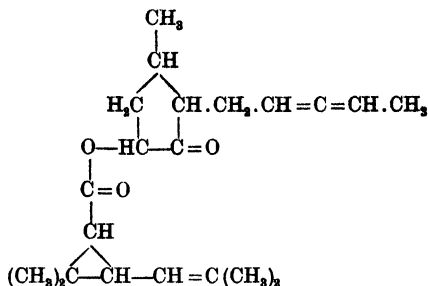
countries is limited only by the price of labour and the cost involved in harvesting the flower-heads which on account of their higher insecticidal value are usually separated from the leaf and stalk. In a paper already published<sup>(1)</sup> it has been demonstrated that *C. cinerariaefolium*, can be grown in England and that the toxicity of the English-grown flowers is equal to that of the material grown abroad. There are, however, several problems that await solution. The area under cultivation might perhaps be extended if flowers of higher poison content could be produced by systematic plant breeding, as this would lower the cost of production per toxic unit. The relationship between methods of cultivation and manuring and toxic content would also repay investigation. In this connection it has been recently suspected<sup>(3)</sup> that under continued artificial cultivation there may be a gradual lowering of the content of poison, leading to loss of value and to the necessity for more frequent replanting. Owing also to the expansion in the general use of pyrethrum, methods of rapidly standardising the product are urgently required.

In order to facilitate the study of these problems, a fairly rapid method of evaluation has become necessary. Two methods suggest themselves, (1) a direct biological determination by testing the effect produced upon suitable insects, and (2) a determination of the toxic constituents by chemical analysis. A suitable biological method for determination of insecticidal values has already been described<sup>(10)</sup> and has been extensively employed in this and other insecticide investigations. The objection to this method in the present case is that it requires considerable technical experience to employ with precision, and the harvesting of *C. cinerariaefolium* in July leaves very little time in this country for biological tests, before colder weather makes it difficult to rear suitable insects in sufficient quantity for adequate trials. A chemical method is therefore desirable.

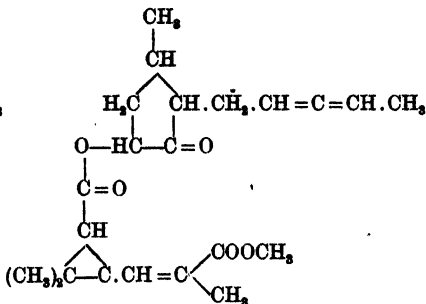
The present paper deals with a study of two methods of chemical analysis and the results obtained are as far as possible compared with a series of biological tests carried out on the same material. In order to obtain a more complete check and to investigate in detail the analytical procedure, the toxic principles have been isolated in a pure state and their insecticidal values determined.

Our knowledge of the constitution of the toxic principles of *Pyrethrum* is mainly due to the classical researches of Staudinger and Ruzicka<sup>(8 a)</sup> who isolated them in a pure state, established their structure, and determined the relative importance of the two constituents in contributing to the

toxicity of the flowers. They gave them the names of Pyrethrin I and II and ascribed to them the following formulae:



Pyrethrin I



Pyrethrin II

Pyrethrin I is therefore an ester of a monocarboxylic acid containing an unsaturated side-chain and a trimethylene ring, and a cyclopentalone derivative with an unsaturated side-chain (pyrethrolone). In pyrethrin II the monocarboxylic acid is replaced by the methyl ester of a dicarboxylic acid of a very similar structure.

Staudinger and Ruzicka<sup>(8b)</sup> ascribe the toxic properties of these compounds to their peculiar structure, small changes in this respect being sufficient to eliminate their insecticidal action. They showed that neither the alcoholic nor the acidic portions of the molecule were insecticidal.

#### PREPARATION OF THE PYRETHRINS.

The pyrethrins were isolated by us by the method of Staudinger and Ruzicka (*loc. cit.*). This process involves extraction with petroleum ether, a preliminary purification of the extract by means of methyl alcohol in the cold, and the removal of fatty and resin acids by shaking out a petroleum ether solution with alkali. From the oleo-resin thus obtained the pyrethrins are condensed with semicarbazide in the cold. The subsequent hydrolysis of the semicarbazone with methyl alcoholic soda splits off the acids, and the semicarbazone of pyrethrolone separates. After recrystallisation the latter is converted to pyrethrolone by mild acid hydrolysis with potassium hydrogen sulphate. The acids are separated by steam distillation, and the volatile acid (monocarboxylic) purified by distillation *in vacuo*. The dicarboxylic acid, isolated from the residue by extraction with ether, is purified by means of its crystalline chloroform compound and partially methylated. The monocarboxylic acid and the monomethyl ester of the dicarboxylic acid are then converted to the acid chlorides by the action of thionyl chloride, and after

distillation *in vacuo* condensed with pyrethrolone in the presence of quinoline to pyrethrin I and II.

Pyrethrin I was distilled *in vacuo* and boiled at 145° C. at 0.05 mm. pressure. Pyrethrin II was not distilled, as it decomposes readily at high temperatures, but was purified by solution in low boiling-point petroleum ether and subsequent cooling in a freezing mixture, which removed a small amount of resinous matter.

#### TOXICITY TESTS WITH PYRETHRINS I AND II.

The method employed for the determination of contact insecticidal values has already been described<sup>(10, 11)</sup> but may be briefly summarised here. *Aphis rumicis* L. (the Black Bean Aphis) has been used as the chief test insect, and large numbers are specially reared for the experiments. In order to standardise the conditions as far as possible, only insects at a certain stage of development (adult wingless females) are taken for the tests, and as the result of considerable experience, successive generations of the aphides at this stage can be obtained during the summer months, which show little individual variation in resistance as judged by the results of many duplicate experiments. Care is taken to rear the insects under uniform conditions, and they are protected from risk of attack by parasites. The spraying apparatus used<sup>(10)</sup> is designed to give percentage mortality figures under strictly controlled and constant conditions with regard to the amount of liquid sprayed, the pressure employed to produce the spray (15 lb. per sq. in.), and the length of time of contact. A large number of tests can be carried out rapidly, and by testing each compound at a number of concentrations, it is possible to draw diagrammatic curves from the results, plotting percentage mortality against concentration, and thus to obtain a convenient means of comparing toxicities.

In making up the mixtures for spraying, small amounts of the pyrethrins were weighed out, dissolved in a little alcohol in a graduated flask and diluted with a 0.5 per cent. aqueous solution of saponin to assist spreading and wetting, both compounds being thus obtained at known concentrations and in a very finely distributed condition. Dilutions were made with 0.5 per cent. saponin solution. Control experiments, many times repeated, have shown that 0.5 per cent. or 1.0 per cent. solutions of saponin are without toxicity to *A. rumicis*.

After spraying, the insects were placed within reach of fresh bean foliage and were examined and classified on the two following days. At each examination counts were made under four headings: "un-

affected," "slightly affected," "moribund," and "apparently dead," and the results are recorded thus in Table I.

Table I. *Toxicities of Pyrethrin I and II to A. rumicis.*

(N=not affected, S=slightly affected, M=moribund, D=apparently dead.)  
Marks allowed for each concentration = ( $\frac{1}{2}$ S % +  $\frac{1}{4}$ M % + D %).

	No. of tests	Concentration in gm./100 c.c.	N %	S %	M %	D %	M and D %	Marks
Pyrethrin I (not dis- tilled)	2	0.05	—	—	—	100	100	100
	2	0.025	—	—	—	100	100	100
	2	0.01	—	—	—	100	100	100
	3	0.005	—	—	—	100	100	100
	3	0.0025	—	—	27	73	100	86.5
	2	0.001	5	10	70	15	85	52.5
	2	0.0005	55	25	10	10	20	21
	1	0.00025	70	30	—	—	0	7.5
Pyrethrin I (after dis- tillation in <i>vacuo</i> )	1	0.05	—	—	—	100	100	100
	1	0.025	—	—	—	100	100	100
	1	0.01	—	—	—	100	100	100
	2	0.005	—	—	20	80	100	90
	2	0.0025	—	—	25	75	100	87.5
	1	0.001	—	30	70	—	70	42.5
	1	0.0005	70	20	—	10	10	15
Pyrethrin II	1	0.05	—	—	50	50	100	75
	3	0.025	—	7	50	43	93	70
	3	0.01	10	6.5	67	16.5	83.5	51.5
	4	0.005	30	22.5	27.5	20	47.5	39
	3	0.0025	53.5	20	—	26.5	26.5	31.5
Pyrethrolone	...	...	Not toxic at a concentration of 0.2 gm. per 100 c.c.					
The monocarboxylic acid				"	"	"	"	
The dicarboxylic acid				"	"	"	"	

For purposes of diagrammatic representation we have used two methods of evaluating these classes. In the first method (*a*) we have expressed the moribund and dead together as a percentage of the total number of insects sprayed at each concentration. In the second method (*b*) we have awarded at each concentration arbitrary valuations or marks by adding to the percentage of dead half the percentage of moribund and a quarter of the percentage of the slightly affected. This method of treatment may be a slight understatement of the values at the higher concentrations. In both methods of computation, the values at the lowest concentrations are only approximate. Those values ranging between 90–40 per cent. are of greatest importance for purposes of comparison.

The results are shown in Diagrams 1 *a* and 1 *b*, in which the values given by both methods of computation are plotted against the concentrations in grammes per 100 c.c. In the case of pyrethrin I, the scale is

ten times that used for pyrethrin II, this procedure enabling the results to be expressed in an area of reasonable dimensions.

An inspection of Table I and Diagrams 1 *a* and 1 *b*, shows that Pyrethrin I is many times more toxic than pyrethrin II, to *Aphis rumicis*.

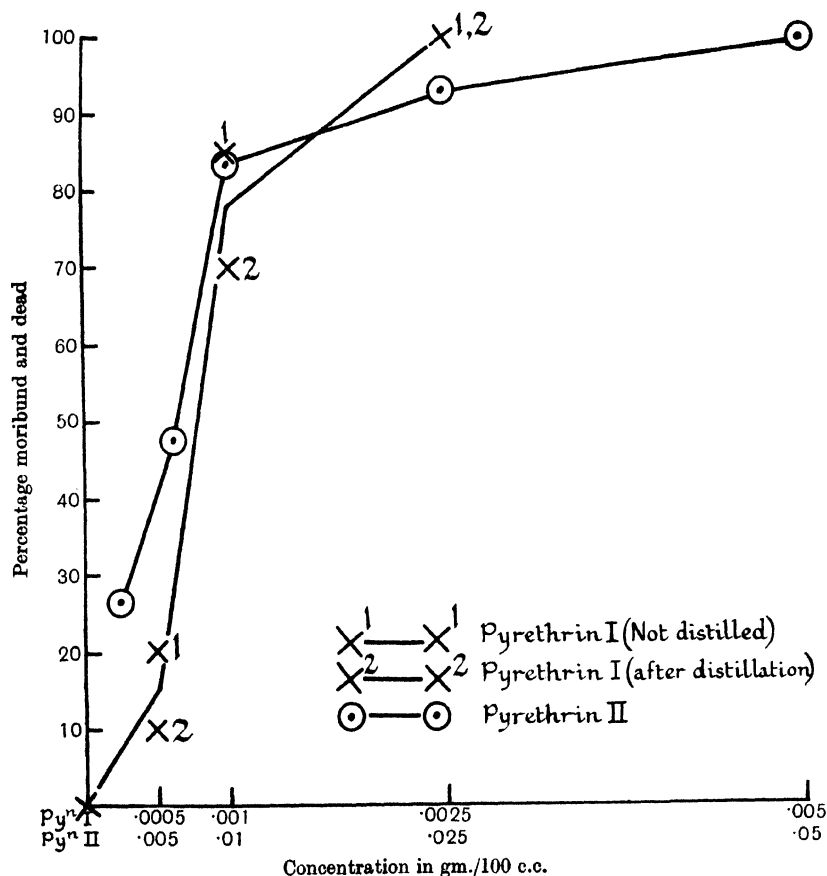


Diagram 1 *a*. Toxicities of pyrethrin I and II to *A. rumicis*. Toxicities evaluated in terms of percentage of moribund, and dead insects.

Staudinger and Ruzicka (8c) found that to cockroaches pyrethrin I was the more toxic and they express the opinion that the insecticidal effect of pyrethrum is almost wholly due to pyrethrin I.

In view of the method of synthesising pyrethrin II, from pyrethrolone and the dicarboxylic acid, there is the possibility that a certain amount of an isomer may be present in the product, which may result in a loss of toxicity. Later work on extracts of the flower-heads to be described

below, indicates, however, that except at higher concentrations, the toxicity of the pyrethrum can be closely correlated with its content of pyrethrin I, thus confirming the lower toxicity of pyrethrin II.

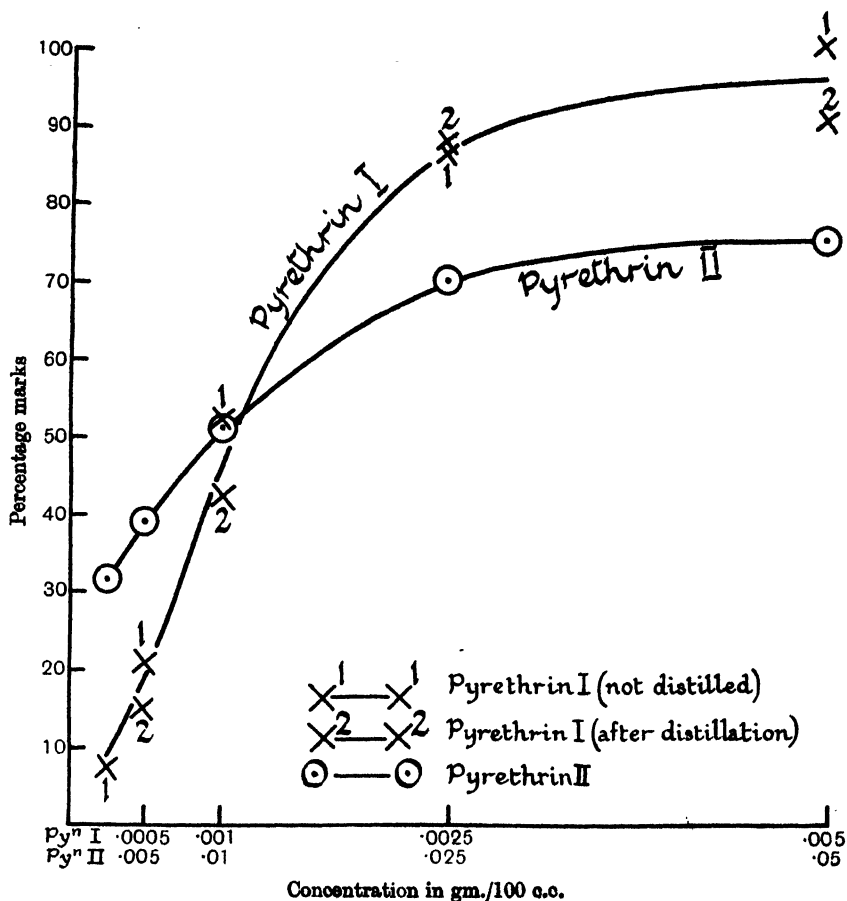


Diagram 1 b. Toxicities of pyrethrin I and II to *A. rumicis*.  
Toxicities evaluated by marks.

Pyrethrin I possesses a higher insecticidal value by contact than any compound tested by us; the only substance which in our experience approaches it in toxicity is rotenone (tubatoxin), the characteristic constituent of the fish-poison, *Derris* (*Deguelia elliptica*) and of White and Black Haiari (*Lonchocarpus* spp.). Calculated from the amount of spray falling on an individual aphid of the size used in our experiments, the lethal dose is approximately  $3 \times 10^{-6}$  to  $6 \times 10^{-6}$  gm. of pyrethrin I, and of this only a small fraction comes into effect.

Pyrethrin II is of a lower order of toxicity than pyrethrin I, but none the less is an exceedingly toxic substance, ranking higher than nicotine in this respect.

The constituent parts of the pyrethrins were not found toxic at any concentrations tested, pyrethrolone, and the mono- and dicarboxylic acids having negligible effects. The toxicity of both compounds is, therefore, due to some critical make-up of the pyrethrin molecule taken as a whole.

#### METHODS OF ESTIMATION OF THE PYRETHRINS.

The drawbacks to the biological method of evaluating pyrethrum, lie in the facts that it is somewhat difficult to obtain a satisfactory supply of suitable insects throughout the year, that it requires biological equipment for breeding and rearing insects in a condition suitable for the work, and that the technique requires time and special training for its proper application. Although a biological method will probably remain the final court of appeal in evaluating such an insecticide, it is nevertheless true that a chemical method, provided it is sufficiently rapid and accurate, is urgently needed. For plant-breeding purposes it should prove invaluable, if from time to time it were backed up by direct tests on insects. The biological method used by us only calls for small quantities of material, which is also a *sine qua non* in the case of an analytical process applied to purposes of plant breeding.

Staudinger and Harder<sup>(9)</sup> have published an account of two chemical methods, which may be described as (a) the acid, and (b) the semi-carbazone method. We have undertaken a critical study of both with the object of making them shorter and of reducing the amount of material required for the analyses.

##### *The acid method.*

Staudinger and Harder<sup>(9)</sup> extracted 500 gm. of the powder with low-boiling petroleum ether, and after evaporating off the solvent extracted the residue with methyl alcohol. Ten per cent. of water was added, and after cooling in a freezing mixture the precipitate of resins and fats was filtered off. The filtrate was then hydrolysed with an excess of methyl alcoholic soda, the methyl alcohol taken off in partial vacuum and the residue extracted with ether. The mixture was then acidified and distilled in steam for two hours, the distillate being titrated with *N*/10 soda. Harder<sup>(2)</sup>, however, recommends the separation of the monocarboxylic acid from the distillate by extraction with petroleum ether, and titrating after evaporating the volatile solvent. From the value for the acid the pyrethrin I content was calculated.

Pyrethrin II was determined in the residue after steam distillation, which after clearing with animal charcoal was extracted with methylated ether in a Kutscher-Steudel apparatus, the extract being afterwards evaporated and titrated. This gave a value for the dicarboxylic acid from which the pyrethrin II content was calculated.

We have attempted to adapt this method for use with very much smaller quantities of material, and for each analysis have used 10 gm. of flowers or 50–100 gm. of stalk.

For an examination of each stage of the analysis an exceptionally toxic sample of the flowers was chosen. Four lots of 10 gm. were completely extracted with petroleum ether and the solvent was taken down in a strong current of  $\text{CO}_2$ , the last traces being eliminated by evacuation in a desiccator. The residues were subjected to the following treatments:

1. The extracts obtained by gentle warming with four lots of 2.5 c.c. of methyl alcohol (purified over caustic soda), were collected in a centrifuge tube, cooled in ice and salt, and centrifuged clear; 10 per cent. of water by volume was added and after cooling in ice and salt, the extract was again centrifuged. The supernatant liquid was poured off from the residue into a 100 c.c. long-necked flask, and the washings of the residue added (two lots of 2 c.c. of alcohol containing 10 per cent. of water). The pyrethrins were estimated by saponification and determination of the volatile and water-soluble acids.

2. Extracted with four lots of 2.5 c.c. of methyl alcohol containing 10 per cent. of water, cooled in ice and salt one hour and centrifuged clear. Treatment then as 1.

3. As 2, but cooling in ice omitted.

4. Extracted with absolute methyl alcohol, cooled under the tap and filtered through a small wad of fat-free cotton wool. Hydrolysis and the remainder of the treatment was afterwards as in 1.

The results were as follows:

Pyrethrin I (%)	(1) 0.57	(2) 0.56	(3) 0.56	(4) 0.59
„ II (%)	0.47	0.49	0.49	0.57

The total residues from 1 were examined further by extraction with alcohol containing 10 per cent. of water and found to contain 0.02 per cent. pyrethrin I and 0.04 per cent. pyrethrin II.

The values for pyrethrin I are in substantial concordance and, although that of pyrethrin II in (4) is higher than in the other cases, there is obviously some risk of loss by adsorption by the flocculent pre-

cipitates produced on adding water to the methyl alcohol extracts. As, moreover, pyrethrin II contributes only in a minor degree to the toxicity of pyrethrum, we have preferred to use pure methyl alcohol for extraction and have omitted the addition of water. This procedure obviates the risk of loss by frothing when the methyl alcohol is taken off *in vacuo*, and shortens and simplifies the process materially.

Tests were made on various samples, to ascertain the effect of omitting the extraction with methylated ether after hydrolysis, with the following results:

Origin of sample Flowers	Extracted		Not extracted	
	Py. I %	Py. II %	Py. I %	Py. II %
Swanley ... (1927)	0.41	0.47	0.41	0.46
Wye ... (1927)	0.37	0.34	0.35	0.32
Harpenden ... (1928)	0.59	0.67	0.59	0.73
	0.58	0.70		
Harpenden stalk (1928)	0.028	0.033	0.031	0.034

The differences between the results are small and within the experimental error, and although most of our tabulated results have been obtained after methylated ether extraction after saponification, this process does not appear to serve any very useful purpose.

*The determination of the monocarboxylic acid and pyrethrin I.* On distilling in steam a sample of pyrethrum extract treated as above, the distillate is acid even after prolonged distillation; one sample (10 gm.) gave the following figures in c.c. for the titration with *N*/50 soda for each 20 c.c. of distillate:

(1) 5.72, (2) 1.6, (3) 1.15, (4) 0.64, (5) 0.51, (6) 0.37.

When an amount of the pure acid equivalent to 10 gm. of a rich sample of pyrethrum is distilled in the same way, 95 per cent. comes over in the first 20 c.c. and practically the whole is recovered in 40 c.c. of distillate. In the case of pyrethrum extracts, therefore, only a portion of the acid in the distillate is the monocarboxylic acid.

It was considered by us for some time that examination of the distillation curve would yield some method of differentiating between the acid associated with pyrethrin I and the other volatile acids in the distillate, but it was found, particularly with a micro-method, that the process could not be controlled with sufficient accuracy to yield data of value on this point. We have therefore employed extraction with low boiling petroleum ether to effect a separation of the monocarboxylic acid. We have found that the pure acid in an amount equivalent to that present in our determinations, distributes itself between petroleum

ether and water in the ratio of 15 to 1. The repeated extraction of one of the distillates from pyrethrum with an equal volume of petroleum ether gave the following titration figures after evaporation of the solvent:

1st extract required 8.8 c.c. *N*/50 soda.

2nd	„	0.4	„
3rd	„	0.1	„
4th	„	0.1	„

These figures show that this method separates the monocarboxylic acid quantitatively, and we have therefore extracted the first 50 c.c. of the distillate twice with an equal volume of petroleum ether and subtracted a blank of 0.2 c.c., as 0.1 c.c. appears to be a constant blank for each extraction. Relatively large amounts of acid insoluble in petroleum ether are present in the distillate; the values obtained with a number of samples of pyrethrum flowers when the distillates were extracted with petroleum ether and titrated with *N*/50 caustic soda gave the following results in c.c.:

Acid soluble in petroleum ether	(1) 6.25	(2) 5.78	(3) 5.96	(4) 6.86
Acid insoluble in petroleum ether	8.95	8.8	6.75	7.2

It is therefore evident that there is no obvious relationship between the titration value of the gross distillate and the amount of the monocarboxylic acid present.

*The determination of the dicarboxylic acid and pyrethrin II.* The dicarboxylic acid is left in solution in the distillation flask. We have shown with the pure acid that prolonged distillation in steam does not lead to any loss. It has therefore been our practice after collecting the first 50 c.c. containing the monocarboxylic acid to continue the steam distillation for another 100 c.c.; this leads to an aggregation of the resinous particles and facilitates subsequent filtration. Staudinger and Harder used animal charcoal followed by filtration for the purpose of clearing the solution. Our experience has been that this leads to loss of the dicarboxylic acid, and if a sufficient quantity of charcoal is used to its complete adsorption. We have therefore added, instead, 0.2 gm. of pure calcium sulphate to the hot liquid in the flask, which is allowed to stand overnight to cool. The calcium sulphate crystallises on cooling, and clears the liquid. We have shown that calcium sulphate neither adsorbs nor combines with the acid. After this treatment the filtered solution is extracted with methylated ether and the dicarboxylic acid in the extract titrated with *N*/50 soda.

For the extraction with methylated ether we have used an automatic

extractor. The apparatus, which is shown in Fig. 1<sup>1</sup>, presents little difficulty in making; it is only necessary to mention that the internal tube has a small pellet of glass sealed on at the bottom to prevent the

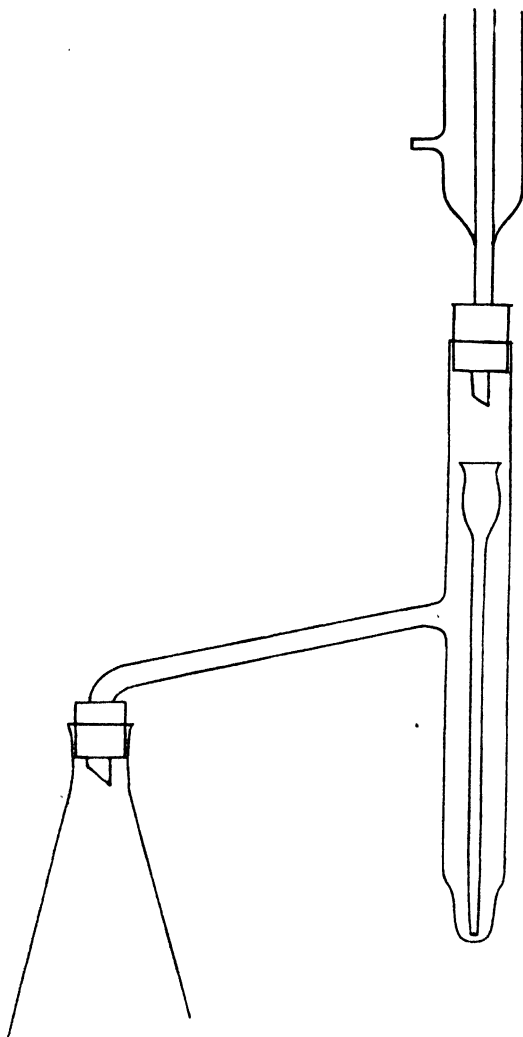


Fig 1. Automatic extractor.

stoppage of the flow of ether, that the hole at the bottom should be constricted to give a fine stream of bubbles, but should not be so small as to become clogged by the deposition of calcium sulphate which takes

<sup>1</sup> The scale of the figure is from one quarter to one fifth actual size.

place as the solution becomes saturated with ether. The ether is boiled in the conical flask and on condensing flows through the inner tube and returns after percolating the aqueous layer.

*Description of acid method.* The procedure finally adopted in the acid method is therefore as follows:

Ten gm. of the ground flower-heads or 50 gm. of the stalk are extracted by low boiling-point petroleum ether (B.D.H. A.R., boiling range below 40° C.) in a Soxhlet extractor heated over a carbon filament lamp; when extraction is complete, the petroleum ether is evaporated to a small bulk in a rapid current of CO<sub>2</sub> with very gentle warming, the evaporation being completed in a vacuum desiccator. The residue is extracted successively with four (or in the case of the stalk, six) lots of 2.5 c.c. of absolute methyl alcohol<sup>1</sup> (free from acid) with gentle warming on a water-bath; each extraction is cooled under the tap, filtered through a small wad of fat-free cotton wool into a long-necked flask of 100 c.c. capacity, the extraction flask being finally rinsed with 2.5 c.c. of cold methyl alcohol. The clear solution is treated with 4 c.c. of *N*/1 caustic soda in methyl alcohol and boiled under a reflux condenser for six to eight hours after which the methyl alcohol is taken off in partial vacuum with gentle warming; a right-angled tube connection to the condenser is used to stop the spray from being carried over. The temperature should not be allowed to rise so high that the alcohol condenses in the connecting tube. The stopper and connecting tube are washed down into the flask, a little water is added to bring the soaps into solution, 6 c.c. of *N*/1 sulphuric acid is added, and the acid liquid distilled in steam. The distillation apparatus used by us was similar to the original Pregl micro-Kjeldahl still<sup>(6)</sup>, except that the rubber connections with the condenser are eliminated so that the glass tube connects directly with a small worm condenser and leads to a receiver marked at the 50 c.c. level. 50 c.c. of distillate are collected for the determination of the volatile acid, and the distillation then continued until a further 100 c.c. have been collected. The first 50 c.c. of distillate is then transferred to a pear-shaped funnel closed with a rubber stopper and is extracted twice with 50 c.c. of low boiling-point petroleum ether which can be conveniently measured out in the receiver. For complete extraction vigorous shaking is necessary, each extract being washed with a small amount of distilled water. After adding 20 c.c. of water to the extract the ether is gently evaporated and the residue titrated with *N*/50 caustic soda using

<sup>1</sup> A high-grade commercial methyl alcohol free from acetone was boiled under reflux with caustic soda for several hours and fractionated.

phenolphthalein. As the acid tends to adhere to the glass it is necessary to wash down the sides of the flask with neutralised alcohol. We have titrated to a distinct pink which after shaking remained for at least one minute<sup>1</sup>.

To the hot residue in the flask, which should not exceed 40 c.c., 0.2 gm. of pure calcium sulphate is added and after standing overnight the solution is filtered into the automatic extractor, already described, through a small wad of fat-free cotton wool, just tight enough to give a gentle flow. The filtrate should be crystal clear. The extraction is carried out with acid-free methylated ether for a period of eight hours, after which 20 c.c. of distilled water are added to the ether extract and the ether gently taken off; the aqueous layer is finally filtered through a loosely packed wad of fat-free cotton wool and the dicarboxylic acid titrated with *N*/50 soda.

The factors to be used are as follows:

$$\begin{aligned} 1 \text{ c.c. } N/50 \text{ alkali} &= \frac{16.8}{5.0} = 3.36 \text{ mg. monocarboxylic acid,} \\ &= \frac{33.0}{5.0} = 6.6 \text{ mg. pyrethrin I,} \\ &= \frac{9.9}{5.0} = 1.98 \text{ mg. dicarboxylic acid,} \\ &= \frac{18.7}{5.0} = 3.74 \text{ mg. pyrethrin II.} \end{aligned}$$

#### *The semicarbazone method.*

Whereas the acid method has presented no great difficulty in adapting to a small scale technique, the determination of the pyrethrin content by means of the semicarbazone required considerable modification. Moreover, the procedure is tedious and gives only an approximate estimation of the sum of the two pyrethrins, and as pyrethrin I is the main toxic constituent of pyrethrum, there is some objection to its use for practical purposes; we have, therefore, only used it to confirm that the amounts of the acids are a true measure of the pyrethrin content.

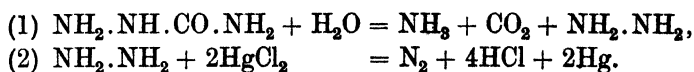
The procedure adopted by Staudinger and Harder (*loc. cit.*) for the semicarbazone method was as follows: They used 500 gm. of the powder and carried out the extraction with low boiling-point petroleum ether and the purification with 90 per cent. methyl alcohol exactly as in the acid method. The oil obtained after evaporation of the solvent was

<sup>1</sup> Further work, to be published separately, has shown that for routine analyses a very rapid assay of pyrethrin I can be made by extracting the plant material with petroleum ether (boiling range 40–50° C.), transferring the petroleum ether solution to the flask used for distilling, hydrolysing with *N*/1 soda in methyl alcohol for one to two hours, acidifying and distilling in steam until two lots of 50 c.c. of aqueous distillate have collected. The petroleum ether distilling is used for the first extraction of monocarboxylic acid, which is titrated without evaporating the solvent.

dissolved in methyl alcohol and treated with an excess of semicarbazide hydrochloride and sodium acetate in solution in a small amount of water. After standing 24 hours the whole was evaporated *in vacuo* to dryness and the residue was washed with water to remove the uncombined semicarbazide. The semicarbazone was then estimated by a determination of the nitrogen in the residue by Wilfarth's<sup>(12)</sup> modification of the Kjeldahl method, in which a mixture of fuming sulphuric and phosphorus pentoxide is used with mercury as a catalyst; the amount of the pyrethrin was then calculated.

*Determination of semicarbazone.* The use of the Kjeldahl method for estimating semicarbazones is open to criticism, as no modification of this method, to our knowledge, ammonifies quantitatively the hydrazo grouping. We have found that pure semicarbazide hydrochloride reacts to the Kjeldahl method to the extent of about 30–40 per cent., but it is probable that mixtures containing other organic compounds (such as the impure pyrethrin semicarbazones) would react more completely, as it is well known that the presence of oxidisable carbonaceous material effects a partial reduction of such nitrogen groupings to ammonia; nevertheless it is apparently quite impossible by the addition of reducing agents to convert hydrazo compounds to ammonia quantitatively. We have therefore abandoned the Kjeldahl method for this purpose.

We have worked out a method suitable for the micro-analysis of semicarbazones by combining and modifying the methods of Rimini<sup>(7)</sup> and of Maselli<sup>(4)</sup>. The essential reactions are as follows:



Rimini hydrolyses semicarbazide and semicarbazones with acid (reaction (1)) and then oxidises the hydrazine with mercuric chloride in alkaline solution (reaction (2)) measuring the nitrogen evolved. Maselli carries out acid hydrolysis and determines either the hydrazine by titration with potassium iodate or the ammonia by making alkaline and distilling. We have carried out the reactions (1) and (2) in one stage by boiling with a hydrochloric acid solution containing mercuric chloride; the semicarbazide is broken down into hydrazine (which is then oxidised to nitrogen) and ammonia, which can be determined readily even in very small amounts. The presence of the mercuric chloride is necessary in the case of semicarbazones in order to oxidise the hydrazine and so prevent its reduction by the ketone formed or by any organic impurities. This reduction was found to occur if semicarbazide hydrochloride was

boiled with hydrochloric acid to which sucrose had been added, as more than one-third of the total nitrogen was found as ammonia.

The time required for the complete hydrolysis of semicarbazide by 15 per cent. hydrochloric acid containing 5 per cent. mercuric chloride is six hours, as the following results with the pure hydrochloric acid show:

After  $1\frac{1}{2}$  hours hydrolysis 28.3 % of total nitrogen recovered as  $\text{NH}_3$

" 4	"	32.8	"	"	"
" 6	"	33.25	"	"	"

In the case of pyrethrin semicarbazones seven hours hydrolysis is therefore allowed.

We have shown that a pyrethrum extract does not interfere with the reaction, by hydrolysing a known amount of semicarbazide to which had been added an approximately equivalent amount of pyrethrum extract; the amount of ammonia found corresponded to 34 per cent. of the total nitrogen of the semicarbazide. The small increase over the theoretical value of 33.3 per cent. is probably due to traces of ammonia produced from the extract.

*Conversion of pyrethrins to semicarbazones.* Apart from the question of determining the amount of semicarbazone, the adaptation of the method of Staudinger and Harder as a micro-determination presents two main difficulties, (1) the complete condensation of minute amounts of ketone with semicarbazide, and (2) the removal of this reagent from the product.

(1) We have examined the conditions necessary for complete condensation. Ten gm. of flower-heads were extracted with low boiling-point petroleum ether; after removing the solvent *in vacuo* the residue was extracted with methyl alcohol and the solution filtered and made up to 10 c.c. For each analysis 4 c.c. (equivalent to 4 gm. of powder) were taken. We have omitted the preliminary precipitation of the methyl alcohol solution with 10 per cent. of water, as extracts purified in this way gave low results. The lower figures are due to the presence of the water and not to the removal of impurities that interfere, for if the water was removed *in vacuo* and the oil taken up in pure methyl alcohol before condensing the same results were obtained as with untreated methyl alcohol extracts. At 25° C. the reaction with semicarbazide appears to be complete in 36 hours, provided the methyl alcohol solution is first concentrated to a volume of  $\frac{1}{2}$ –1 c.c. The results reach a maximum after 36 hours and then remain constant within the

experimental error; this is shown by the following figures for one sample:

24 hours at 25° 0.80 % pyrethrins.			
36	„	1.24	„
48	„	1.09	„
72	„	1.17	„

Four days standing at room temperature gave approximately the same results, but a longer period gave higher figures. We believe this to be due to resinification which renders the subsequent removal of the excess of semicarbazide very difficult.

(2) After condensation, it is necessary to evaporate off the alcohol as the semicarbazone does not crystallise out and the addition of water produces an emulsion which cannot be separated. In spite of the small amount of material we have found great difficulty in washing out the free semicarbazide from this residue, even when it is spread out in a very fine film. In order to do this we have found it necessary after each washing to take up the residue in ether and evaporate down again to a film. A dilute solution of acetic acid and sodium acetate was used for washing. The semicarbazide in the washings was then titrated with potassium permanganate, and it was found necessary to repeat the process of solution, evaporation, and washing, three times; further repetition continued to give a small constant titration figure in the washings but this was found to be due to some soluble organic substance, as a pyrethrum extract which had not been treated with semicarbazide gave a similar result. We have also used an alternative method by taking up the residue (after evaporating off the alcohol) with ether and washing the ether solution with water in a separating funnel. As part of the semicarbazone does not dissolve in ether it is necessary to filter the aqueous washings. Similar results were obtained by the two methods as is shown by the following results of three analyses of the same sample.

Washed 3 times as a film in the tube 1.00 % pyrethrins.

Washed 6 times as a film in the tube 1.10 % pyrethrins.

Ether solution washed in a separating funnel 1.02 % pyrethrins.

*Description of the semicarbazone method.* Our procedure in the semicarbazone method is then as follows: 10 gm. of the powdered flower-heads are extracted completely with low boiling-point petroleum ether and the residue, after evaporation of the solvent, is extracted with absolute methyl alcohol, in exactly the same way as for the acid method.

The extracts are filtered successively into a small measuring cylinder and brought to a volume of 10 c.c. The solution is mixed and 4 c.c. aliquots are pipetted into a hard glass test-tube. 30 mg. of semicarbazide hydrochloride and 50 mg. of sodium acetate are added and the solution is concentrated to about 0.5 c.c. This is done by inserting a rubber stopper having a capillary tube reaching to the bottom of the liquid and a side-tube which is connected to a pump; the test-tube is kept at a temperature of 25° C. and the alcohol can then be readily removed by evacuation. A small glass bead, to act as a spray-trap, was inserted in the side-tube and held in place by constricting the tube on either side. At the finish a few drops of methyl alcohol are poured into the side tube, to free it from any deposit carried up by the spray, and so manipulated as to wash down the capillary as well. The test-tube is corked and placed in a bath at 25° for 36 to 48 hours. The methyl alcohol is then evaporated to dryness by connecting to a pump and evacuating without the use of a capillary. Two methods of washing have been employed. (a) A little ether is added to the residue and the solution is then evaporated to a film by tilting and rotating the test-tube which is prevented from cooling by warming with the hand. The last traces of ether are removed in a good vacuum. The film should cover half the length of the test-tube. Twenty c.c. of a solution containing 0.1 per cent. acetic acid and 1 per cent. sodium acetate are poured carefully into the tube and left to stand 10 minutes; the film should be completely covered. The solution is then filtered through a small plug of cotton wool and the test-tube rinsed out with about 2 c.c. of water. The film is then remade by solution in ether and evaporation and is again washed, the whole process being carried out three times in all. The small residue on the filter is returned to the test-tube by pouring through two drops of methyl alcohol and 1 c.c. of ether. The alcohol and ether are evaporated *in vacuo*. Five c.c. of a 15 per cent. solution of hydrochloric acid containing 5 per cent. of mercuric chloride are heated and poured through the filter into the test-tube. (b) The alternative and more rapid method of washing is as follows: after complete removal of the methyl alcohol, the residue is twice extracted with 5 c.c. of ether and the ether solution poured into a small separating funnel. The residue in the test-tube is washed four times with 10 c.c. of water, the washings are successively filtered through a cotton wool plug into the separating funnel and used to wash the ether solution with gentle rotation. The ether solution is washed twice more with small amounts of water, returned to the test-tube and evaporated. Five c.c. of the 15 per cent. hydrochloric acid solution

containing 5 per cent. mercuric chloride are heated and used to wash into the test-tube the insoluble material left in the separating funnel and the filter, the cotton-wool plug in the latter being previously loosened.

The test-tube is fixed to a small reflux condenser and after the addition of a little pumice the solution boiled for seven hours. Ammonia is then determined by the method adopted by Pregl in his revised micro-Kjeldahl method (6).

The solution used for rendering alkaline was prepared by diluting a 40 per cent. solution of caustic soda with an equal volume of a saturated solution of sodium thiosulphate which is necessary for the decomposition of the mercury ammonium complex. *N*/50 acid was used for absorbing the ammonia, and *N*/50 alkali for titration.

For the mixed pyrethrins which are present in nearly equal proportions we have taken 350 as the approximate mean molecular weight, 1 c.c. of *N*/50 acid being then equivalent to 70 mg. of the combined pyrethrins.

#### RESULTS OF ANALYSIS.

In Table II the results obtained by the acid method and also in certain cases by the semicarbazone method are given in duplicate. The results give some idea of the agreement to be obtained by parallel determinations by the acid method. In addition, the table shows that there is reasonable agreement between the sum of the percentages of pyrethrin I and II as obtained by the acid method and this value as determined by the semicarbazone method.

*The correlation between the contents of pyrethrin I and II.* The data in Table II indicate that there is an apparent connection between the values for the two pyrethrins. We are indebted to Dr Wishart of the Rothamsted Statistical Dept. for kindly analysing the figures for the first eight samples in the table. He finds the correlation coefficient between pyrethrin I and II in these samples to be positive and to have a value of 0.85. This is definitely significant and although the number of samples is small and the seed from which they were grown was of the same origin, there is a distinct suggestion of a close connection in the derivation of the two poisons in the plant, either from some parent body or by some chemical reaction common to the synthesis of both or it may be that one compound is derived from the other.

*The relationship between size of flower-heads and the content of the pyrethrins.* For a long time there has been an opinion that the closed

and half-closed flowers of pyrethrum are more toxic to insects than fully opened ones, although critical examination has thrown doubt on this view.

Table II. *Results of analysis of various samples of pyrethrum.*

Origin of sample	Acid method			Semi-carbazone method	Weight of 100 flower-heads gm.	Standard deviation
	Pyrethrin I	Pyrethrin II	Total			
	1927 Harvest					
Harpenden	0.35	0.36	0.71	0.71	13.91	0.27
	0.36	0.35	0.71	0.74		
Reading	0.39	0.33	0.72	—	11.65	0.31
Scilly Isles	0.43	0.46	0.89	—	12.87	0.31
	0.46	0.46	0.92			
Seale Hayne	0.28	0.31	0.59	0.60	13.95	0.44
				0.58		
Swanley	0.41	0.46	0.87	0.90	9.62	0.26
	0.41	0.48	0.89	0.86		
Wisley	0.39	0.42	0.81	0.82	11.22	0.29
	0.39	0.41	0.80	0.86		
Worcester	0.46	0.57	1.03	1.10	9.59	0.29
	0.45	0.54	0.99	1.00		
Wye	0.35	0.32	0.67	0.74	9.66	0.16
	0.38	0.33	0.71	0.71		
1926 Harvest						
Scilly Isles	0.60	0.49	1.09	1.13*		
	0.59	0.58	1.17			
Wye	0.37	0.34	0.71	0.77		
Origin of sample	Acid method			Semi-carbazone method	Petr. ether extract %	Pyrethrin I and II % of petr. ether extract
	Pyrethrin I	Pyrethrin II	Total			
	1928 Harvest					
Harpenden grown						
Plot 20 c						
Flowers	0.58	0.70	1.28	1.18	5.44	23.6
	0.59	0.70	1.29	1.14		
Flowers and Stalk	0.25	0.32	0.57	—	2.95	20.0
	0.26	0.35	0.61			
Stalk	0.031	0.034	0.065	—	1.13	5.6
	0.028	0.033	0.061			
Plot 17 c						
Flowers	0.47	0.59	1.06	—	5.75	18.6
	0.47	0.61	1.08			
Flowers and stalk	0.23	0.31	0.54	—	3.36	16.0
	0.032	0.036	0.07	0.09		
Stalk					1.07	6.5

\* Mean of six determinations.

The data in Table II for the first eight samples were used for testing the dependence, if any, of the pyrethrin values upon the size of flower-heads. Random samples were taken in a suitable way and the weight

of ten lots of ten heads of each sample determined, giving the weight of each hundred heads. The standard deviations of the mean, calculated from these figures, show a certain amount of variation between the samples in point of size and uniformity. The data were subjected to statistical analysis by Dr Wishart, who found the correlation between the weight per 100 flower-heads and percentages of pyrethrin I to be  $-0.527$  and that for pyrethrin II  $-0.543$ . The correlation while reasonably large and negative cannot be regarded as significant for so small a number as eight samples.

The data are too meagre to settle the matter finally, but taking individual samples and comparing them with each other we find how uncertain any choice resting upon a selection by size of flower-heads would be and that the source of origin is at least as important. For example, although in the Worcester and Seale Hayne samples, the ratio of the pyrethrin contents is as 1.0 to 0.6 and the weight per 100 heads as 9.6 to 14 respectively, in the case of the Wye and Harpenden samples, whereas the proportionality in the weight of heads is the same (9.6 to 14), that of the pyrethrin contents is as 0.7 to 0.7. Moreover, the loss of yield, ensuing from harvesting the smaller-sized heads of the closed and half-closed flowers as against taking the crop when they are fully open, outweighs in economic importance any advantage likely to accrue from the possibility of the former possessing a larger amount of the two pyrethrins.

The loss on heating at  $105^{\circ}\text{C}$ . was determined, but, as this did not vary amongst the samples to an extent sufficient to modify the deductions drawn from the analytical results, we have preferred to express our data on the air-dried samples.

*The pyrethrin content of flowers, stalk, and mixed stalk and flowers.* Table II gives data for the pyrethrin content of two samples of stalk, in addition to those of the flowers and the mixed flowers and stalk from two beds, 17 c and 20 c at Harpenden; in addition, it contains the percentage amounts of the petroleum ether extracts and also their content of pyrethrin. These samples were carefully taken and, after air-drying in the shade, each was divided into three portions; in one the proportion of flower-heads to stalk was determined, the stalk and flowers being ground separately; the second portion was ground as a whole, while a third portion was kept for reference. The pyrethrin content of the stalk is between one-fifteenth and one-twentieth of that of the flowers. Although we have no exact data, the few tests carried out biologically indicated a similar relationship in the toxicity of the stalk and flowers

respectively, and experiments carried out in previous years on the plants from these beds indicated that the stalk is in general less than one-tenth as toxic as the flower-heads.

The percentage weights of petroleum ether extracts of the flowers, of the mixture of flowers and stalk and of the stalk are very different, the stalk for both beds 17 *c* and 20 *c* giving only about one-fifth of that of the flowers. The contents of pyrethrin were therefore calculated on these extracts and on that of the mixed stalk and flower. The counterbalancing effect of the small weight of extract from the stalk upon the percentage of pyrethrins is considerable and as a result an extract of the mixture of stalk and flower contains in the case of plot 17 *c* 16.6 per cent. pyrethrin against 18.6 per cent. in the flowers, and in the case of 20 *c* 20.0 per cent. against 23.6 per cent. In the case of the mixture the total amounts of pyrethrin extracted per unit weight of plant material is of course about one-half that of the flowers.

The results of the analyses of the flowers, the stalk and a mixture of the two afford a means of testing the validity of the method for use with material containing both stalk and flowers. In the sample 20 *c* the mixture of flowers and stalk contained approximately 44 per cent. of flowers; a calculation from the percentages of pyrethrin I gave 40.5 per cent. and from pyrethrin II 45 per cent. The mixture from bed 17 *c* contained 46 per cent. of flowers; the analysis gave 46 per cent. calculated from pyrethrin I and 49 per cent. from pyrethrin II. The presence of stalk in the sample did not, therefore, introduce any complicating factor in the analysis.

#### COMPARISON OF ANALYTICAL RESULTS WITH TOXICITY TESTS.

The final criterion of the analytical method lies in the degree of concordance with the results of toxicity tests with insects. Quantitative investigations with biological material are by the nature of the case less accurate than chemical analyses, and the determination of results depends largely on the quality of the biological material, which may fluctuate, and upon the care taken in observing the effects produced. In order, therefore, that there should be entire freedom from bias, the two sides of this investigation were kept entirely independent of each other and no comparison was made between the results until both were complete. The biological trials were carried out in the following way: 10 gm. of each sample of ground flowers were allowed to soak in a known amount of absolute alcohol for some days, dilutions were then made by pipetting off the clear supernatant solution

into 0.5 per cent. solutions of saponin in water. Control tests showed that the insects used could tolerate much higher proportions of alcohol and saponin than were used in these experiments. The insects were then sprayed in the manner already described and a short time later duplicate experiments were carried out. As in the case of the biological tests with the pyrethrins which were made within a few days of these experiments, an observation of the results was made after 24 and again after 48 hours. The toxic effects persisting at the end of 48 hours were taken for purposes of comparison, and the categories and methods of computing employed for the pyrethrins were used for evaluating the results. The results are given in Table III.

In Table III, in addition to the expression of the concentrations in weight of flower-heads, we have given the percentage of pyrethrin I present at each concentration. The data in this table are placed under three headings—A, B and C, for in addition to the samples cropped in 1927 and tested in 1928, there are figures given for the crop from two stations taken in 1926 and tested in 1927, and also a certain amount of preliminary data for the flowers grown at Swanley and Wye in 1927 and tested shortly after receipt in that year. Comparisons of the data should only be made within their appropriate groups as duplicate tests should be done within as short an interval as possible.

The toxic effects of the various samples in Group A can be graded in the following order: (1) Worcester—the most toxic sample; (2) Scilly Isles, Swanley, Wisley, Reading, Harpenden—not quite so toxic; and (3) Seale Hayne—least toxic. In the case of the Wye samples difficulties were encountered in placing the sprayed insects in their appropriate categories. We are readily able to separate the Worcester sample from the Seale Hayne and these two are the samples containing the largest and least amount, respectively, of pyrethrin. The intermediate samples cannot be separated from each other with any degree of precision, but in toxic values they definitely come between these two, as they also do in percentage of pyrethrin.

Group B of this table contains two samples whose toxicity data have been dealt with more fully elsewhere<sup>(1)</sup>. It was there stated that it was highly probable that the differences found in the toxicity of the Scilly Isles and Wye samples of 1926 were significant. We have determined the content of pyrethrin in both samples and found that of the Scilly Isles sample to be materially higher than that of the Wye sample.

Group C contains data of a preliminary nature, the results of one set of experiments carried out in August 1927 on two samples harvested

Table III. *Toxicities to A. rumicis of pyrethrum flowers grown at different stations.*

(N—not affected, S—slightly affected, M—moribund, D—apparently dead.)

Marks awarded to each concentration = (S % + ½ M % + D %).

Sample and origin	Concentration								Marks
	In terms of part of plant gm./100 c.c.	As pyrethrin I gm./100 c.c.	N %	S %	M %	D %	M and D %		
Group A*. Harvest, 1927. Tested, 1928.									
Harpenden (1)	0.5	0.0018	—	—	30	70	100	85	
Pyrethrin I = 0.355 %	0.4	0.0014	—	—	40	60	100	80	
„ II = 0.355 %	0.3	0.0011	—	—	43	57	100	78.5 (?)	
	0.2	0.0007	25	20	45	10	55	37.5	
	0.1	0.00036	85	10	5	—	5	2.5	
	0.05	0.00018	100	—	—	—	0	0	
Reading (2)	0.5	0.00195	—	—	20	80	100	90	
Pyrethrin I = 0.39 %	0.4	0.0016	—	—	50	50	100	75	
„ II = 0.33 %	0.3	0.0012	—	5	75	20	95	58.5	
	0.2	0.00078	20	15	65	—	65	36	
	0.1	0.0004	75	10	5	10	15	12.5	
	0.05	0.0002	90	10	—	—	0	2.5	
	0.025	0.0001	100	—	—	—	0	0	
Scilly Isles (3)	0.5	0.0022	—	—	20	80	100	90	
Pyrethrin I = 0.445 %	0.4	0.0018	—	—	40	60	100	80	
„ II = 0.46 %	0.3	0.0013	—	5	55	40	95	69	
	0.2	0.0009	25	5	55	15	70	44	
	0.1	0.0004	60	25	10	5	15	16	
	0.05	0.0002	80	20	—	—	0	5	
Seale Hayne (4)	0.5	0.0014	—	—	70	30	100	65	
Pyrethrin I = 0.28 %	0.4	0.0011	—	10	90	—	90	47.5	
„ II = 0.31 %	0.3	0.00084	—	25	70	5	75	46	
	0.2	0.00056	70	10	20	—	20	12.5	
	0.1	0.00028	90	—	—	10	10	10	
	0.05	0.00014	80	20	—	—	0	5	
Swanley (5)	0.5	0.002	—	—	—	100	100	100	
Pyrethrin I = 0.41 %	0.4	0.0016	—	—	40	60	100	80	
„ II = 0.47 %	0.3	0.0012	10	—	55	35	90	62.5	
	0.2	0.0008	35	10	55	—	55	30	
	0.1	0.0004	100	—	—	—	0	0	
Wisley (6)	0.5	0.0019	—	—	—	100	100	100	
Pyrethrin I = 0.39 %	0.4	0.0016	—	—	40	60	100	80	
„ II = 0.415 %	0.3	0.0012	5	5	85	5	90	49	
	0.2	0.00078	45	10	30	15	45	32.5	
	0.1	0.00039	55	10	35	—	35	20	
	0.05	0.0002	100	—	—	—	0	0	
Worcester (7)	0.5	0.0022	—	—	—	100	100	100	
Pyrethrin I = 0.46 %	0.4	0.0018	—	—	27.5	72.5	100	87	
„ II = 0.415 %	0.3	0.0014	—	2.5	25	72.5	97.5	85.5	
	0.2	0.0009	10	—	55	35	90	62.5	
	0.1	0.00045	50	15	35	—	35	21	
	0.05	0.00022	90	10	—	—	0	2.5	
Wye (8)	0.5	0.0018	—	—	50	50	100	75	
Pyrethrin I = 0.375 %	0.4	0.0014	—	—	50	50	100	75	
„ II = 0.325 %	0.3	0.0011	15	25	55	5	65	38.5 (?)	
	0.2	0.0007	15	40	25	20	45	42.5 (?)	
	0.1	0.00036	80	—	5	15	20	17.5	
	0.05	0.00018	100	—	—	—	0	0	
Group B*. Harvest, 1926. Tested, 1927.									
Scilly Isles	0.5	0.0029	—	—	—	100	100	100	
Pyrethrin I = 0.59 %	0.25	0.00145	—	—	—	100	100	100	
„ II = 0.53 %	0.1	0.00058	—	—	40	60	100	80	
	0.05	0.00029	—	10	75	15	90	55	
	0.025	0.00014	—	40	60	—	60	40	
Wye	0.5	0.00185	—	—	—	100	100	100	
Pyrethrin I = 0.37 %	0.25	0.0009	—	10	5	85	90	90	
„ II = 0.34 %	0.1	0.00037	20	5	30	45	75	61	
	0.05	0.00018	65	15	10	10	20	19	
Group C*. Harvest, 1927. Tested, 1927.									
Swanley	0.5	0.00205	—	—	—	100	100	100	
Pyrethrin I = 0.41 %	0.25	0.0010	—	—	—	100	100	100	
„ II = 0.47 %	0.1	0.0004	—	—	30	70	100	85	
	0.05	0.0002	—	20	30	50	80	70	
Wye	0.5	0.0019	—	—	—	100	100	100	
Pyrethrin I = 0.375 %	0.25	0.0009	—	—	—	100	100	100	
„ II = 0.325 %	0.1	0.00037	—	30	10	60	70	72.5	
	0.05	0.00019	50	20	—	30	30	35	

\* As insect resistance does not remain constant from year to year comparisons of results can only be made within each group.

a few weeks earlier; they are included because of the unsatisfactory data for the Wye sample when tested in 1928. The Swanley sample is slightly the more toxic, although the differences are scarcely significant, and it is noticeable that the content of both pyrethrins is higher in the Swanley than in the Wye sample.

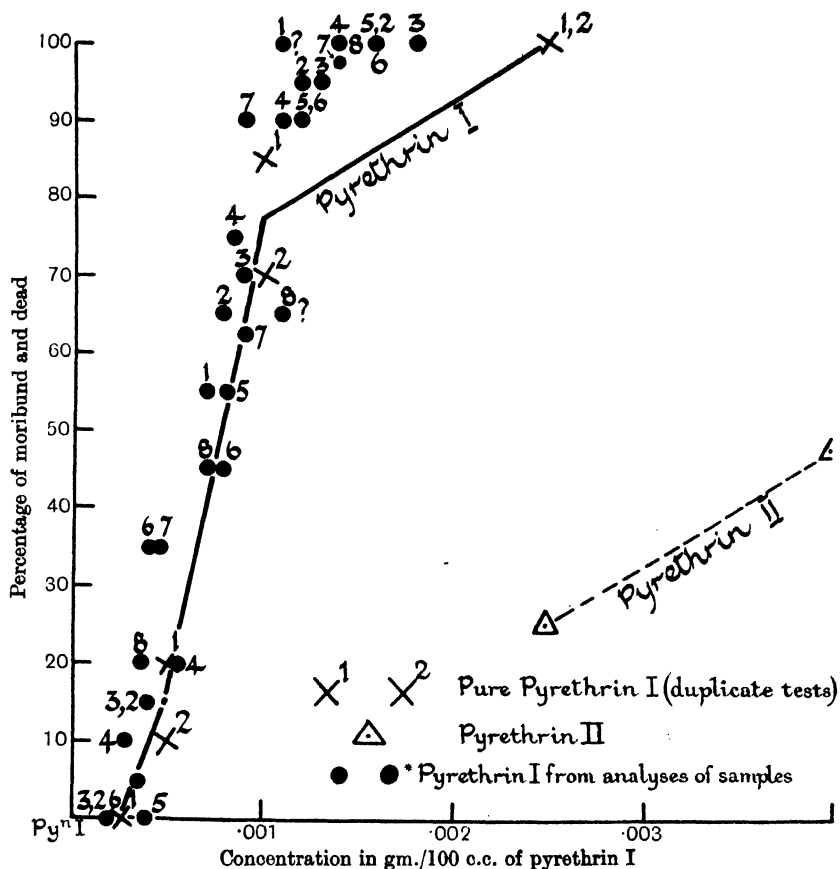


Diagram 2a. Toxicity to *A. rumicis* of pyrethrin I in the isolated state, and as calculated from analysis of flower-heads. Toxicity evaluated in terms of percentage of moribund and dead insects. The numbers to the black circles correspond to numbers of samples in Table III.

Pyrethrin I was considered by Staudinger and Ruzicka to be mainly responsible for the insecticidal effect of pyrethrum; the data given above for the toxicity of the isolated poisons lead to the same conclusion. It appeared a practical method of testing the acid method of analysis, to plot the toxicity values for the above samples against the actual con-

centrations of pyrethrin I (calculated from the dilutions and the percentage amounts of pyrethrin I in the samples), upon the same diagram as the toxicity values of a specimen of the pure poison itself, and on the same scale. This would determine the degree of concordance. We have

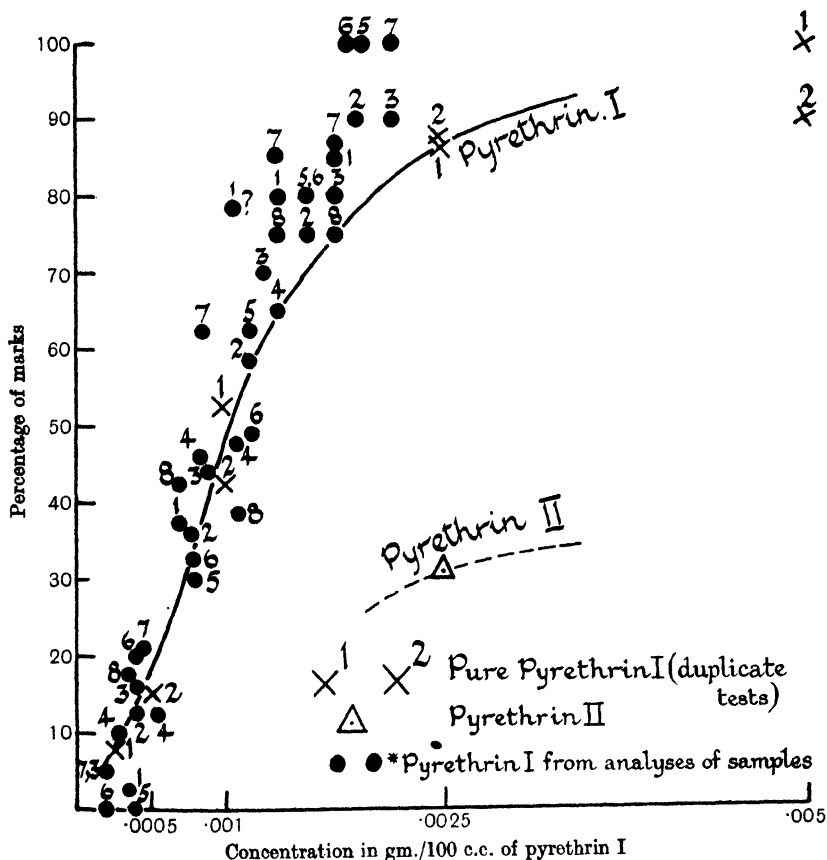


Diagram 2b. Toxicity to *A. rumicis* of pyrethrin I in the isolated state, and as calculated from analysis of flower-heads. Toxicity evaluated by marks. The numbers to the black circles correspond to numbers of samples in Table III.

done this in Diagrams 2a and 2b, the numbers on the diagrams corresponding to the numbers given to the samples in Table III.

Owing to the rather arbitrary nature of evaluation, we have in these diagrams used both the methods of computing toxicity referred to on p. 270. In Diagram 2b we have drawn the shortest smooth curve through the points for pyrethrin I which are marked by crosses. Taking

into account the minute concentrations which are effective, the points obtained for the various samples of pyrethrum fall significantly close to the curves indicating the toxicity of pyrethrin I. Towards the upper part of the curves there is an indication that the samples are more toxic than their content of pyrethrin I would suggest; it should, however, be noted that the curve for pyrethrin I itself may possibly ascend more steeply than shown owing to the rather wide separation of the points, but it is also probable that at these higher concentrations pyrethrin II plays a definite part in influencing the toxicity of the samples. On a diagram on this scale the toxicity of pyrethrin II is represented by only one point (shown by the triangle). We consider that the degree of concordance between the two sets of results is sufficient to demonstrate the value of the acid method, and particularly the determination of pyrethrin I, as a means of evaluating pyrethrum.

Staudinger and Ruzicka (*loc. cit.*) have indicated the possibility of a small amount of the acids being combined with other alcohols than pyrethrolone. If this were so, a lack of concordance between the analytical results and toxicity would be expected. The data presented, and the close agreement of the results given by the semicarbazone method, show that in our samples such compounds can only be present in traces, and their effect can probably be discounted. The possibility, however, suggests that from time to time the semicarbazone method or a direct estimation of toxicity should be used as confirmative tests.

#### THE EFFECT OF EXTERNAL CONDITIONS UPON THE CONTENT OF PYRETHRIN.

Pyrethrum being a perennial crop, it is a matter of importance to know how the toxic properties vary with the age of the plant. The data given in Table III gives a little preliminary information as to the variation in pyrethrum from year to year, *e.g.* the crop taken in Harpenden in 1927 has a percentage content of pyrethrin of 0.7 per cent., whereas in 1928 it has increased in plot 17 *c* to 1.0 per cent. and in plot 20 *c* to 1.28 per cent. The pyrethrin content of flowers taken in the Scillies in 1926 averages 1.1 per cent. but in 1927 the percentage had dropped slightly to 0.85 per cent. These variations are significant, and they cannot be related to differences in soil, or to the age of the plants as the effects for the two stations are in the reverse order. It may be tentatively suggested, that the meteorological conditions prevailing during a critical portion of the growth period have a bearing on the production of the toxic compounds in the plant. In Harpenden, at any rate, the

years 1927 and 1928 were climatically very different, 1927 being cold and wet with little sunshine, and 1928 dry and warm with considerable sunny periods.

With respect to soil conditions it is usually considered that poor calcareous soils are most suitable for pyrethrum. The plants we have tested were from a wide range of soils but our data are not sufficient to yield definite information as to the type of soil most suitable for this plant.

#### CONCLUSIONS.

It is evident from the data given, that, to certain insects, pyrethrin I is the most highly toxic contact poison at present known, and in our experience rotenone (tubatoxin) is the only compound that approaches it at all closely in toxicity. The results of our experiments agree too with those of Staudinger and Ruzicka in showing pyrethrin II to be less toxic than pyrethrin I and, although we find a relatively larger difference than they did, the discrepancy is almost certainly due to the wide differences both in the insects and technique employed. The important fact emerges that the contact insecticidal properties of pyrethrum are almost entirely due to the presence of pyrethrin I, and the aim of plant breeding should be to increase its amount.

Pyrethrum has the considerable advantage over most potent insecticides of being comparatively harmless to man, and there should be in consequence a large field of usefulness before it. Its use at present is circumscribed by its specificity of effect, certain insects being very resistant to it, and to the supposed readiness with which toxicity is lost. There is, however, no reason why its use should not be extended and this might be greatly facilitated if flowers of higher toxicity could be produced by systematic plant breeding.

For the latter purpose some suitable method of determining the percentage of poisons is necessary which should be reasonably rapid and simple. Hitherto most of the methods suggested have depended upon the determination of some value in no way correlated with, or a measure of, the amount of the toxic principle present. No method can be entirely satisfactory that does not definitely assess the amount of the poisons themselves. The micro-methods described above are adaptations of Staudinger and Harder's macro-methods, and care has been taken to test their correlation with toxicity determinations as accurately as possible. Bearing in mind the unique toxicity of pyrethrin I, the close accordance between the toxicities of the flower-heads, when calculated

to their content of pyrethrin I, and that of the isolated poison affords a striking confirmation of the validity of the results as given by the acid method. A further independent check is provided by the semicarbazone method, which depends on the estimation of the alcoholic constituent of the pyrethrins and has given results in substantial agreement with those obtained from a determination of the acidic constituents.

A suitable analytical method, in addition to its value for plant breeding for higher poison content, is likely to prove of use for standardising extracts. Again, the employment of the stalk of pyrethrum has up to the present been regarded as a questionable practice, as it contains a much smaller proportion of the toxic principles than the flowers; it nevertheless provides a potential source of the pyrethrins, if suitable methods of extraction could be devised. The data in Table II show that the percentage of petroleum ether extract of the stalk is much less than that of the flowers, and that in consequence, the petroleum ether extract of mixed stalk and flowers contains only a slightly lower percentage of pyrethrin than the extract of the flowers themselves. The weight of extract per unit weight of plant material being less in the case of the mixture, larger amounts of the mixed stalk and flowers than of the flowers would be required to give the same weight of dry extracts. The economic significance of this procedure would depend very largely on the increased cost of the transport, handling and extracting a larger bulk of material, but to some extent this would be offset by the saving of labour in detaching the flowers from the stalk, especially if a mechanical means of harvesting could be devised.

Whether the methods described can be used for detecting adulteration, can only be determined by further experience. The range of pyrethrin I content, even in samples of flowers grown from the same batch of selected seed, is variable and may possibly depend upon the meteorological conditions of the season, even when grown in the same bed. The percentage of pyrethrin is therefore not a constant for even genuine samples of the flowers. Combined with the methods elaborated by McDonnel, Roark, LaForge and Keenan<sup>(5)</sup> it may, however, prove itself of great value for purposes of detecting sophistication, for if the latter is suspected and a large sample is available, the examination of the acids and the determination of their constants should help in the detection of the most skilful adulteration.

It is important to realise, however, that after long exposure to damp conditions pyrethrum powder loses its toxicity and a genuine sample may be devoid of insecticidal value. Whether or not the chemical methods

outlined above will make it possible to detect such loss of toxicity, is a matter for further investigation.

We have used for this work samples of flowers grown in different localities but all raised from selected seed of the same origin<sup>1</sup>; considerable variation, therefore, was not to be expected. The biological tests were able to separate the samples with considerable precision into three classes of high, low and medium toxicity, but were unable to detect smaller differences. The chemical method was found to put the samples into the same categories and, in addition, was able to detect differences not distinguishable by the biological method.

Such small differences, as were found between many of the samples, are of little interest in practice, and it is important for the plant breeder to realise that samples grown from the same seed on the same soil can vary within fairly wide limits according to the season. The methods outlined should prove useful in any attempt to correlate toxicity with meteorological conditions, and in addition should be of value in studying the effect of soil conditions, manuring and cultivation upon the content of pyrethrin, and the useful duration of the plantation. These aspects of the problem of the extended production of pyrethrum call for further investigation.

The work here described forms parts of a co-operative investigation on English-grown pyrethrum as an insecticide (see 1), between the Plant Pathological Laboratory of the Ministry of Agriculture and the Rothamsted Experimental Station. We are indebted to the following for supplying additional material: South Eastern Agriculture College, Wye; Seale Hayne Agricultural College, Newton Abbot; The Horticultural College, Swanley; Research Station, East Malling; Research Station, Long Ashton; University of Reading; University College of South Wales, Aberystwyth; Royal Horticultural Society, Wisley; Experimental Station, Scilly Isles; Dept. of Agricultural Education, Worcester; Farm Institute, Sparsholt; Isle of Ely Demonstration Plot.

### SUMMARY.

1. (a) Pyrethrin I and II have been isolated by the method of Staudinger and Ruzicka from the insecticidal plant Pyrethrum (*Chrysanthemum cinerariaefolium*). Both are shown to be highly toxic to the insect *Aphis rumicis*.

(b) Pyrethrin I was found to be the most toxic substance so far tested by us and, as it was about ten times as toxic to these insects as pyrethrin II, it is concluded that it is mainly responsible for the contact insecticidal value of pyrethrum.

<sup>1</sup> More recent work has indicated that the acid method is applicable to unselected seed from another source.

2. Two micro-analytical methods of determining the pyrethrin content are described. (a) By means of the acids after hydrolysis. (b) By means of the semicarbazone. They are given on pp. 278, 282.

3. The analytical results obtained for a series of pyrethrum samples agreed with their observed insecticidal properties to *Aphis rumicis*.

4. Comparisons of the pyrethrin contents, as estimated, with the results of direct toxicity experiments both on the pyrethrum samples and the pure pyrethrins, confirm the validity of the analytical methods.

5. There was a significant and positive correlation, in the samples tested, between the amounts of pyrethrin I and II.

6. Insufficient data are available to show a significant correlation between the size of flower-heads and the content of poison, or to draw conclusions as to the effect of external conditions such as soil, weather or age of bed.

#### REFERENCES.

- (1) FRYER, J. C. F., TATTERSFIELD, F. and GIMINGHAM, C. T. *Ann. App. Biol.* **15**, 423, 430 (1928). (Rothamsted Memoirs, **14**.)
- (2) HARDER, H. Dissertation, Techn. Hochschule, Zurich, **54**, 63 (1927).
- (3) KAZANETSKI, N. *Compt. Rend. Acad. d'Agric. France*, **30**, 1080 (1927).
- (4) MASELLI, C. *Gazzetta*, **35**, **1**, 267 (1905); *Journ. Chem. Soc.* **88**, A 2, 560 (1905).
- (5) McDONNELL, C. C., ROARK, R. C., LAForge, F. B. and KEENAN, G. L. *U.S. Dept. Agric. Dept. Bull.* **824**, 17 (1926).
- (6) PREGL, F. (trans. Fyfe, E.). *Quantitative Organic Micro-analysis*, pp. 96 and 99. Publ. Churchill (1924).
- (7) RIMINI, E. *Atti R. Accad. Lincei*, (5), **12**, **II**, 376 (1903); *Journ. Chem. Soc.* **86**, A 2, 207 (1904).
- (8) STAUDINGER, H. and RUZICKA, L. (a) *Helv. Chem. Act.* **7**, 177 (1924).  
(b) *Helv. Chem. Act.* **7**, 450 (1924).  
(c) *Helv. Chem. Act.* **7**, 449 (1924).
- (9) STAUDINGER, H. and HARDER, H. *Ann. Acad. Scient. Fennicae*, A, **29**, No. 18 (1927).
- (10) TATTERSFIELD, F. and MORRIS, H. M. *Bull. Ent. Res.* **14**, Pt. 3, 223 (1924). (Rothamsted Memoirs, **12**.)
- (11) TATTERSFIELD, F., GIMINGHAM, C. T. and MORRIS, H. M. *Ann. App. Biol.* **12**, 61 (1925). (Rothamsted Memoirs, **12**.)
- (12) WILFARTH, H. *Chem. Centralbl.* **1**, 113 (1885); *Journ. Chem. Soc.* **48**, 837 (1885).

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## PYRETHRIN I AND II.

### THEIR ESTIMATION IN PYRETHRUM (*CHRYSANTHEMUM CINERARIAEFOLIUM*). II.

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#### INTRODUCTION.

WE have recently described two chemical methods for the determination of the poisons in the insecticidal plant pyrethrum (*C. cinerariaefolium*), based on the researches of Staudinger and his co-workers whose results we were able to confirm. Working with pyrethrum grown in England from Swiss seed, we obtained results which could be correlated closely with the observed toxicity of the samples and of the pure poisons themselves.

Staudinger and Ruzicka<sup>(3)</sup> isolated from pyrethrum of European origin two active principles, named by them pyrethrin I and II, to which they ascribe the whole of the insecticidal action and whose structures they determined. The Japanese workers Fujitani<sup>(2)</sup> and Yamomoto<sup>(6)</sup>, however, arrived at different conclusions as to the nature of the active principles and this has led to the suggestion<sup>(4)</sup> that different poisons may be produced by the same plant, *Chrysanthemum cinerariaefolium*, grown in Japan and Europe. Although this hypothesis is highly improbable, it nevertheless seemed important to establish the validity of the analytical process, not only for European pyrethrum, but also for pyrethrum grown from Japanese seed, as in the event of failure it would not be possible to propose the method as a general one.

Of the two methods already mentioned, the acid method is the more convenient but it is nevertheless a somewhat lengthy process, taking several days to complete. As it had been shown that of the two poisons, pyrethrin I is mainly responsible for the toxicity, it was decided to

explore the possibility of making a rapid evaluation of pyrethrum by estimating pyrethrin I alone by an abbreviated method.

This paper deals with the results of the evaluation, by both chemical and biological means, of pyrethrum grown from Swiss and Japanese seed and includes in addition a study of a rapid approximate method for the chemical assay of pyrethrin I.

#### COMPARISON OF PYRETHRUM GROWN FROM SWISS AND JAPANESE SEED.

During 1928 a considerable number of tests was carried out on the toxicities of the flowers derived from Swiss seed and grown on plots laid down at stations in different parts of the country. Unfortunately, however, towards the latter part of the summer of that year a shortage of suitable insects, particularly *Aphis rumicis*, made it impossible to test adequately the samples grown from Japanese seed. Samples grown in 1926 from Swiss and Japanese seed had been tested in the previous year (1927) in a number of toxicity trials, full particulars of which are published elsewhere (1), and as these samples were still available we have determined in them the content of pyrethrin I and II, by means of the acid method previously described (5). The data are given in Table I.

Table I. *Analyses of Pyrethrum Flowers.*

Station and derivation of seed	Full acid method		Short acid method	Toxicities to <i>A. rumicis</i>	
				Highest conc. giving under 50 % moribund and dead.	Lowest conc. giving over 50 % moribund and dead.
	Pyrethrin I %	Pyrethrin II %	Pyrethrin I %	Gm. flowers/ 100 c.c.	Gm. flowers/ 100 c.c.
<i>Crop 1926</i>					
Scillies. Swiss	0.59	0.53	0.60	—	0.025 (60 %)*
Wye. Swiss	0.36	0.32	—	0.05	(75 %)
Swanley. Japan.	0.30	0.44	0.33		(80 %)
Harpندن. Japan.	0.29	0.50	0.27		(70 %)
Harpندن. Swiss	0.28	0.34	—		(75 %)
Sparsholt. Japan.	0.26	0.28	0.27	(20 %)	(70 %)
E. Malling. Japan.	0.20	0.24	0.19	0.1	(30 %)
Wye. Japan.	0.18	0.30	0.15		(100 %)
<i>Crop 1927</i>					
Worcester. Swiss	0.45	—	0.43	—	—
Scillies. Swiss	0.44	—	0.43	—	—
Swanley. Swiss	0.41	—	0.38	—	—
Wisley. Swiss	0.39	—	0.35	—	—
Wye. Swiss	0.36	—	0.36	—	—
Seale Hayne. Swiss	0.28	—	0.26	—	—

\* The figures in brackets indicate the observed mortalities at the concentrations given.

For purposes of comparison of the analytical results with the toxicity data, we have in the table represented briefly the insecticidal values of

the different samples in the following manner. The samples were tested (1), p. 430) at concentrations, in terms of flowers, of 0.5, 0.25, 0.1, 0.05, and 0.025 gm. per 100 c.c. of spray fluid. Statistically, the concentrations giving 50 per cent. of moribund and dead insects are the most suitable for purposes of comparison, but in view of the difficulties of interpolation, we have stated the highest of the concentrations tested giving *below* 50 per cent. and the lowest giving *over* 50 per cent. of moribund and dead insects.

A consideration of the table shows that for the 1926 crop the samples can be arranged in three groups according to their toxicities and that this grouping can be correlated with the content of pyrethrin I. Thus, the Scillies (Swiss) sample contains 0.59 per cent. and is significantly more toxic than the others, while in the second group of medium toxicity is included a number of samples, derived from both Japanese and Swiss seed, and containing from 0.26 to 0.36 per cent. of pyrethrin I. In the third and least toxic group are placed the two lots of flowers grown from Japanese seed at East Malling and Wye, and containing 0.20 and 0.18 per cent., respectively, of pyrethrin I. In addition to the difficulty of reducing toxicity data to an expression by two figures, the above method of representation can only be regarded as approximate owing to the wide separation of concentrations tested; but it should be stated that a more complete examination of the full data, given by Fryer, Tattersfield and Gimingham (1), p. 430), gives general confirmation for the deductions drawn. It is, therefore, hardly a matter of doubt that the acid method is as suitable for testing flowers from Japanese seed as it is for those grown from Swiss seed, and it seems almost certain that the poisons are identical in the two cases.

#### SHORT METHOD FOR DETERMINING PYRETHRIN I.

The acid method of analysis takes several days to complete and the number of operations required is somewhat large. Seeing that pyrethrin I has been shown to be much the more important poison of the two (5), it was considered advisable to attempt to devise a more expeditious method for its determination. Even if this method were only approximately correct, it would be of great use in sieving out good from poor samples and so materially lessening the labour of differentiating between a large number of different specimens. The longer method could then be limited to the analysis of this smaller group. The following method has accordingly been applied to a number of samples previously analysed by the longer method.

10 gm. of the ground pyrethrum are extracted in a Soxhlet apparatus by means of petroleum ether (boiling range 40 to 50° C.), which is kept vigorously boiling over a carbon-filament lamp. The extraction is continued until the ether draining over is colourless. The petroleum ether solution which should have a volume of approximately 50 c.c. is then poured into a long-necked flask of 100 c.c. capacity, subsequently used for distillation, the extraction flask being rinsed once with a little petroleum ether, 4 to 5 c.c. of *N*/1 caustic soda in methyl alcohol added and the mixture vigorously refluxed on the water-bath for 1½–2 hours. The mixture is then acidified with *N*/1 sulphuric acid and distilled in steam in the apparatus previously described (5). Petroleum ether distils first and until it is completely removed, a flame is not placed under the distillation flask. To prevent risk of fire the worm-condenser is attached to the receiver by means of a cork with two holes, one of which contains a glass tube with a length of indiarubber tubing to carry any inflammable vapour away from the flame. Distillation is continued until 50 c.c. of aqueous distillate stand below the petroleum ether in the receiver, after which another flask is attached and distillation continued until a further 50 c.c. have distilled. The whole of the first distillate is then transferred to a fairly large separating-funnel and vigorously shaken for one minute, the aqueous layer is separated and the petroleum ether layer, after washing once with water, is run off into a flask containing 20 c.c. of water, to which a few drops of alcohol and phenolphthalein have been previously added together with just enough alkali to render the liquid a faint pink. Titration is carried out with *N*/50 soda until the aqueous layer is distinctly alkaline after vigorous shaking in the corked flask. The second 50 c.c. of distillate is added to the first aqueous fraction (which has already been extracted once), vigorously shaken in a separating-funnel with 50 c.c. of petroleum ether, the washed petroleum ether layer added to the titration flask and the titration finished as before; very little additional *N*/50 soda is usually required. After deducting a blank which should be determined for the petroleum ether (about 0.2 c.c. *N*/50 soda), the monocarboxylic acid and pyrethrin I content can be calculated, the following factors being used:

$$\begin{aligned} 1 \text{ c.c. } N/50 \text{ alkali} &= \frac{1.68}{50} = 3.36 \text{ mg. monocarboxylic acid,} \\ ,, &= \frac{3.30}{50} = 6.6 \text{ mg. pyrethrin I.} \end{aligned}$$

Table I includes data obtained by this method and indicates how close are the agreements with the figures obtained by the longer process. Thus, while the method gives results of sufficient accuracy for most

purposes, it can be carried out in a few hours and on a small amount of material. So far, however, we have not found it possible to determine the water-soluble acid in the residue from the distillation, but as pyrethrin II is less toxic than pyrethrin I, this is not a serious omission.

#### SUMMARY.

1. The acid method previously described has been used to evaluate samples of pyrethrum derived from both Swiss and Japanese seed, with equally successful results.

2. A rapid method for the evaluation of pyrethrum by a determination of pyrethrin I is described.

#### REFERENCES.

- (1) FRYER, J. C. F., TATTERSFIELD, F. and GIMINGHAM, C. T. *Ann. App. Biol.* (1928), **15**, 3, 423-430. (Rothamsted Memoirs, XIV.)
- (2) FUJITANI, J. *Arch. exp. Path. Pharm.* (1909), **61**, 47.
- (3) STAUDINGER, H. and RUZICKA, L. *Helv. Chim. Acta* (1924), **7**, 177.
- (4) STAUDINGER, H., RUZICKA, L. and REUSS, E. *Ann. Acad. Sci. Fenn. A* (1927), **29**, 17, 7.
- (5) TATTERSFIELD, F., HOBSON, R. P. and GIMINGHAM, C. T. *J. Agric. Sci.* (1929), **19**, 2, 266. (Rothamsted Memoirs, XIV.)
- (6) YAMOMOTO, R. *Chem. Zentr.* (1920), **3**, 517; (1926), **1**, 693.

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# THE TOXICITY OF CERTAIN SULPHUR COMPOUNDS TO *SYNCHYTRIUM ENDOBIOTICUM*, THE FUNGUS CAUSING WART DISEASE OF POTATOES

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(With 8 Text-figures.)

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## INTRODUCTION.

EARLIER experiments have shown that the toxic action of sulphur on the winter sporangia of *Synchytrium endobioticum* in soil varies considerably under different conditions both of season and of soil type(9, 10). There is evidence from the Rothamsted field(9, 10) and pot experiments(3) and from the pot experiments of Weiss(11) which suggests that

high soil acidity such as may arise from the oxidation of sulphur to sulphuric acid may alone be sufficient to kill the fungus, but the degree of acidity<sup>1</sup> required is too great for fertility. Certain of the results obtained (9, 3), however, suggest that sulphur has a second mode of toxic action which is effective at much lower acidities under certain unknown conditions. Researches dealing with the oxidation of sulphur in soil<sup>2</sup>, considered in conjunction with purely chemical investigations on inorganic sulphur compounds<sup>3</sup>, suggest that a variety of compounds may be formed during the course of the oxidation of sulphur to sulphuric acid, and it is possible that one or more of these may be responsible for this second toxic action. If some form of sulphur is ever to serve as a practical means of controlling wart disease in the soil it is more likely to be by means of this second type of toxic action than by raising the soil acidity. It was decided, therefore, to attack the problem of the variability of the action of sulphur as a soil fungicide by determining which of the compounds, at all likely to be formed when sulphur is added to soil, are toxic to the fungus, as a preliminary to determining the conditions under which such a compound might be formed in the soil. Since those compounds which are more toxic than sulphuric acid are the most likely ones to contribute to the solution of the problem, sulphuric acid was taken as the standard with which to compare the toxicities of the other compounds.

#### EXPERIMENTAL.

A. *Chemical*. The compounds that have been tested are arranged schematically in Fig. 1 in order of the degree of oxidation of sulphur and are placed as far as possible under the oxides from which they may be considered to be derived. The formulae of compounds, the existence of which has not been proved, are placed within square brackets. All the compounds were tested for purity, whether they had been prepared specially for the work or had been obtained already prepared (see Appendix I).

B. *Biological*. Winter sporangia of the fungus were obtained fairly free from other organic matter by removing the outer parts of ripe decaying warts, pressing them through fine muslin and centrifuging in

<sup>1</sup> About pH 3.4 according to the Rothamsted experiments, and pH 3.9 according to Weiss (11).

<sup>2</sup> Of especial interest are the papers of Guittonneau (5, 6) and Guittonneau and Keiling (7), the first of which gives a key to the extensive literature on the subject.

<sup>3</sup> The present position of our knowledge of these compounds is summarised by Bassett and Durrant (2).

[S O]	[S <sub>4</sub> O <sub>6</sub> ]	[S <sub>2</sub> O <sub>3</sub> ]	[S <sub>3</sub> O <sub>6</sub> ]	S O <sub>2</sub>	[S <sub>2</sub> O <sub>5</sub> ]	S O <sub>3</sub>	[S <sub>2</sub> O <sub>7</sub> ]
<b>Pentathionic acid.</b> $\text{H}_2\text{S}_5\text{O}_6$ 	<b>Tetrathionic acid.</b> $\text{H}_2\text{S}_4\text{O}_6$ 		<b>Trithionic acid.</b> $\text{H}_2\text{S}_3\text{O}_6$ 		<b>Dithionic acid.</b> $\text{H}_2\text{S}_2\text{O}_6$ 	<b>Sulphuric acid.</b> $\text{H}_2\text{SO}_4$ 	<b>Persulphuric acid.</b> $\text{H}_2\text{S}_2\text{O}_8$ 
<b>Thiosulphuric acid.</b> $\text{H}_2\text{S}_2\text{O}_3$ 							
<b>Sulphoxylic acid.</b> $[\text{H}_2\text{SO}_2]$ $\text{HO-S-OH}$		<b>Hydro-sulphurous acid.</b> $[\text{H}_2\text{S}_2\text{O}_4]$ 		<b>Sulphurous acid.</b> $\text{H}_2\text{SO}_3$ 			

Fig. 1. Formulae of compounds tested, arranged according to the state of oxidation of the sulphur.

water 15 times for 25 seconds. Portions about 3 mm. in diameter of the damp sporangial material containing large numbers of sporangia were treated with 3 c.c. of the solution, the toxicity of which was to be estimated. After the required periods of exposure, usually 24 hours and 10 days, the sporangia were well washed and their viability tested. As they cannot yet be made to germinate in sufficient numbers in a reasonably short period of time, an indirect method of testing their viability, and so of estimating the toxicity of the compounds, was used. This depends on the differential staining in an aqueous solution of acid fuchsin

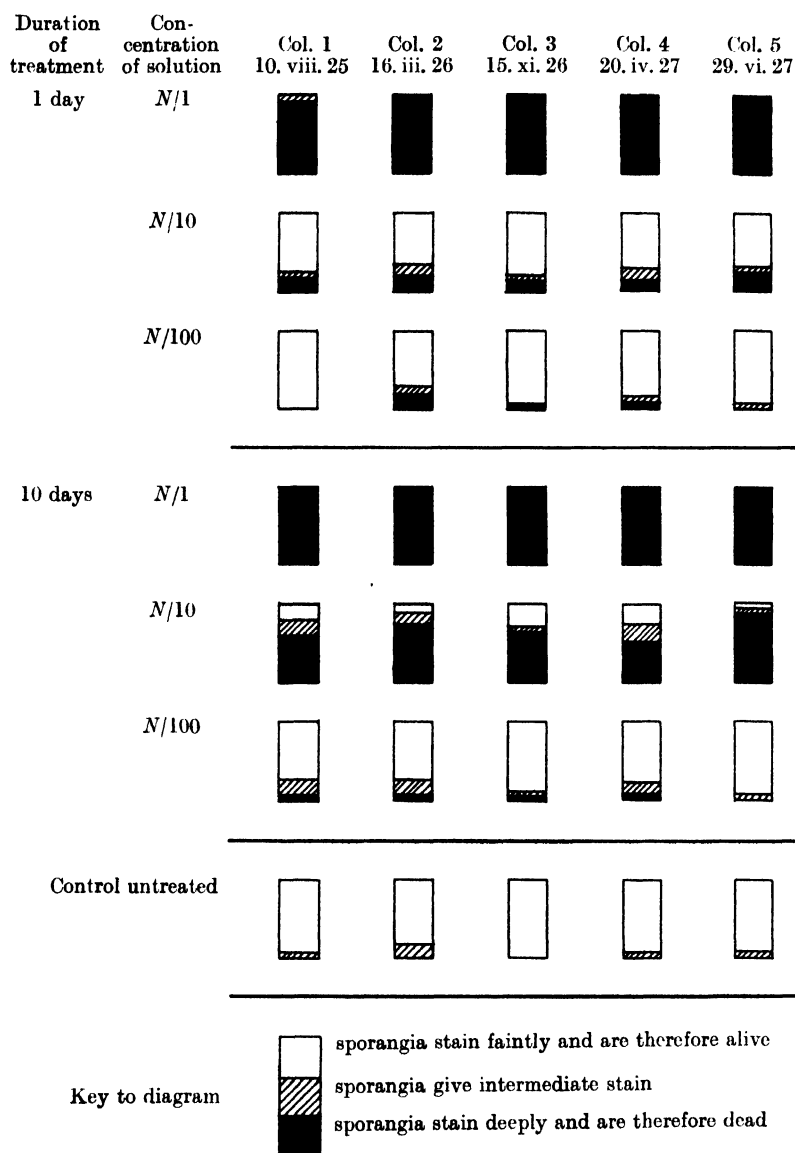


Fig. 2. Variation in toxicity observed at different times with different samples of sporangia treated with sulphuric acid.

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of the contents of dead and living sporangia and has been described in a previous paper<sup>(1)</sup>, where evidence of the reliability of the method is brought forward. The sporangia were mounted in 2 per cent. aqueous acid fuchsin under a coverslip which was gently pressed so as to expel the sporangial contents while under microscopic observation. Three counts of 20 sporangia were made for each test, and the numbers were recorded of (1) those which stained rapidly and deeply, as do dead sporangia, (2) those which stained faintly and slowly like living sporangia, or (3) those which were regarded as intermediate. The numbers of sporangia falling into each of these groups after treatment with the different sulphur compounds gave a measure of their toxicity.

### COMPARISON OF TOXICITIES.

#### *Standard for comparison. Sulphuric acid.*

Sulphuric acid has been taken as the standard with which to compare the toxicities of all the other compounds tested. A comparison of the results obtained from five similar tests with sulphuric acid, carried out at different times over a period of nearly two years, is shown in Fig. 2. The degree of variation shown includes that existing between different samples of sporangia at different times, together with any subjective observational variation<sup>1</sup> in placing the line of demarcation between the three groups, a process which requires some experience. A test with sulphuric acid was always carried out with each new batch of sporangia.

Sulphuric acid at the end of one day is completely toxic in normal solution and only slightly so in decinormal. At the end of 10 days about three-quarters of the sporangia are killed in decinormal solution.

#### *Sulphuric, Dithionic and Sulphurous Acids, and their Neutral Alkali Salts.*

The neutral alkali salts of sulphuric, dithionic and sulphurous acids exerted little, if any, toxic action, suggesting that the  $\text{Na}$ ,  $\text{K}$ ,  $\text{SO}_4$ ,  $\text{S}_2\text{O}_6$  and  $\text{SO}_3$  ions into which these salts are dissociated are non-toxic in neutral solution (Fig. 3).

The acids themselves were completely toxic (*i.e.* all the sporangia dead) in 10 days in normal<sup>2</sup>, and partially so in decinormal solution (Fig. 3). The toxicities of these three acids were approximately equal when compared at the same normality. This coincidence suggests that

<sup>1</sup> The viability tests were all carried out by one worker, *i.e.* M. D. Glynne.

<sup>2</sup> Sulphur dioxide is insufficiently soluble in water for it to be possible to make up a normal solution, so only decinormal and more dilute solutions were tested.

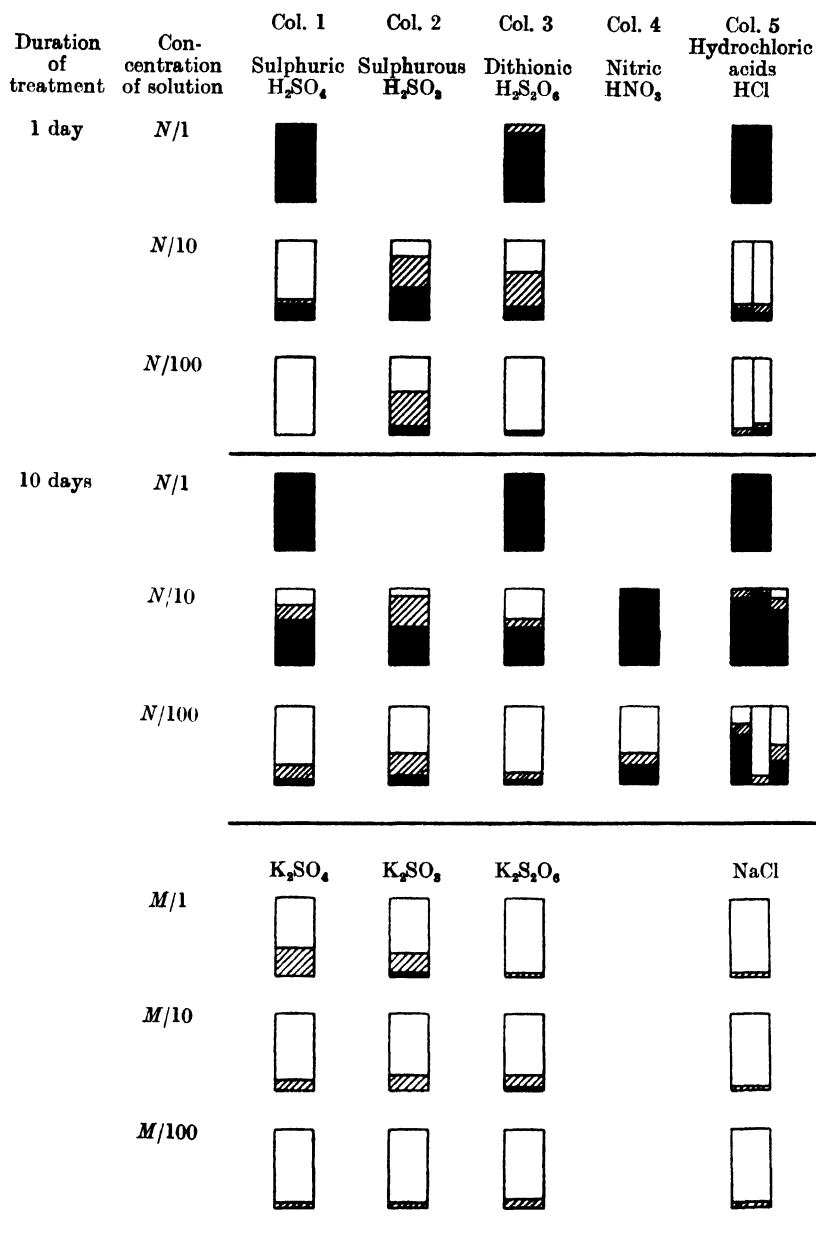


Fig. 3. Toxicities of sulphuric, dithionic, sulphurous, hydrochloric and nitric acids and their salts. (Three different observations are indicated for hydrochloric acid 10 days and two for hydrochloric acid one day.)

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the  $\text{SO}_4$ ,  $\text{S}_2\text{O}_6$  and  $\text{SO}_3$  ions are also non-toxic in acid solution and that the equal hydrogen-ion concentrations of solutions of the same normality is the cause of their equal toxicities, in other words, that these three acids owe their toxicities mainly to their hydrogen-ion concentrations.

If this deduction be valid, then no acid should be less toxic than sulphuric when compared at the same hydrogen-ion concentration, and if an acid is of greater toxicity than sulphuric acid it would suggest either that its anion is toxic or that a toxic impurity is present in the solution.

For instance, none of the samples of hydrochloric or nitric acids tested had a toxicity less than that of sulphuric acid. (The toxicity figures obtained for hydrochloric acid at different times varied to an unusual degree but the lowest value is probably nearest the truth for the pure acid. The greater toxicity of the other two is probably due to the small amounts of free chlorine which so often occur in the concentrated acid.)

The conclusion is further strengthened by the fact that no acid tested in the course of this work was less toxic than sulphuric acid and by the fact that trithionic, tetrathionic and pentathionic acids were found to be of the same degree of toxicity as sulphuric acid (see Fig. 4, p. 176).

If the above acids owe their toxicities mainly to their hydrogen-ion concentration, then it follows that high acidity alone can kill the sporangia. The  $\text{pH}$  value of  $N/10$  sulphuric acid, which in 10 days does not kill all the sporangia, is about 2, a value far below any found in fertile soil; even the value 3 for  $N/100$  acid, which is of very low toxicity, is too low for fertility in soil and is definitely lower than the critical value found in pot experiments, viz. 3.4. Possibly this enhanced toxicity in the soil is due to some indirect effect of the acidity on the soil such as the liberation of toxic salts, *e.g.* those of manganese.

It has however been shown that the total effect of soil acidity does not account for the toxicity of sulphur under all conditions, so that another cause remains to be found.

### *Polythionic Acids.*

Evidence had been obtained by one of us<sup>1</sup> that pentathionate is formed in soils to which sulphur has been added. The polythionic acids, in particular pentathionic acid, have been suggested by Young (12)

<sup>1</sup> W. A. Roach.

as the cause of the general fungicidal action of sulphur. The toxicities of these compounds are therefore of special interest.

The sulphur content of equimolecular solutions of sulphuric, trithionic, tetrathionic and pentathionic acids varies in the ratio of 1 : 3 : 4 : 5. In comparing the toxicity of sulphur in different chemical combinations it is necessary to test solutions containing equal quantities of sulphur. As the hydrogen ion has been shown to be toxic, all these solutions must also have the same hydrogen-ion concentration. These two ends are attained by adding to sulphuric acid of the requisite concentration a sufficient quantity of a neutral salt of the acid to be tested to supply a quantity of sulphur equal to that already contained in the sulphuric acid.

To take the polythionic acids as an example, to 1 litre of normal sulphuric acid is added  $\frac{1}{2}$  gm. molecule of barium trithionate  $\text{Ba}_2\text{S}_3\text{O}_6$ , or  $\frac{1}{3}$  gm. molecule barium tetrathionate  $\text{Ba}_2\text{S}_4\text{O}_6$ , or  $\frac{1}{4}$  gm. molecule barium pentathionate  $\text{BaS}_5\text{O}_6$  respectively. The barium in each solution is precipitated, taking with it an equivalent amount of sulphate, but the normality of the solutions in respect to total acidity is unaltered. Thus the normalities of the three solutions are  $2N/3$  (in regard to  $\text{H}_2\text{SO}_4$ ) +  $N/3$  (in regard to  $\text{H}_2\text{S}_3\text{O}_6$ ),  $3N/4$  (in regard to  $\text{H}_2\text{SO}_4$ ) +  $N/4$  (in regard to  $\text{H}_2\text{S}_4\text{O}_6$ ), and  $4N/5$  (in regard to  $\text{H}_2\text{SO}_4$ ) +  $N/5$  (in regard to  $\text{H}_2\text{S}_5\text{O}_6$ ), respectively. Since all of these acids are strong ones their hydrogen-ion concentrations will not vary sufficiently in the above series to cause any variation in toxicity detectable by the method employed, so that these solutions have approximately the same hydrogen-ion concentration and contain equal quantities of sulphur in the various chemical combinations. They vary in their contents of " $\text{SO}_4$ " ions but evidence that these are non-toxic has been brought forward.

Such solutions are all normal in regard to acidity and so may be designated  $N/1$ , but the symbol  $'H/1$ , representing as it does 1 gm. equivalent of hydrogen ions per litre, is perhaps more suited to the present purpose. They all contain  $\frac{1}{2}$  gm. atom of sulphur per litre and so are conveniently represented in this respect by the symbol  $S/2$ ; combining these two symbols we have  $'H/1, S/2$ . When the above solutions are 10 times diluted they will be represented by the combined symbol  $'H/10, S/20$  and when diluted 10 times again  $'H/100, S/200$ , and so on.

The three polythionic acids themselves were of the same order of toxicity as sulphuric acid (Fig. 4, cols. 1, 2, 5, 7). Neutral solutions of their alkali salts (Fig. 4, cols. 4, 6, 8) were non-toxic. Sodium trithionate solution as tested in the first instance was almost completely toxic in 10 days in  $S/2$  solution and slightly toxic in  $S/20$  solution (col. 3). The solution became slightly acid on standing; this toxicity however was not found when the solution was carefully kept neutral by adding

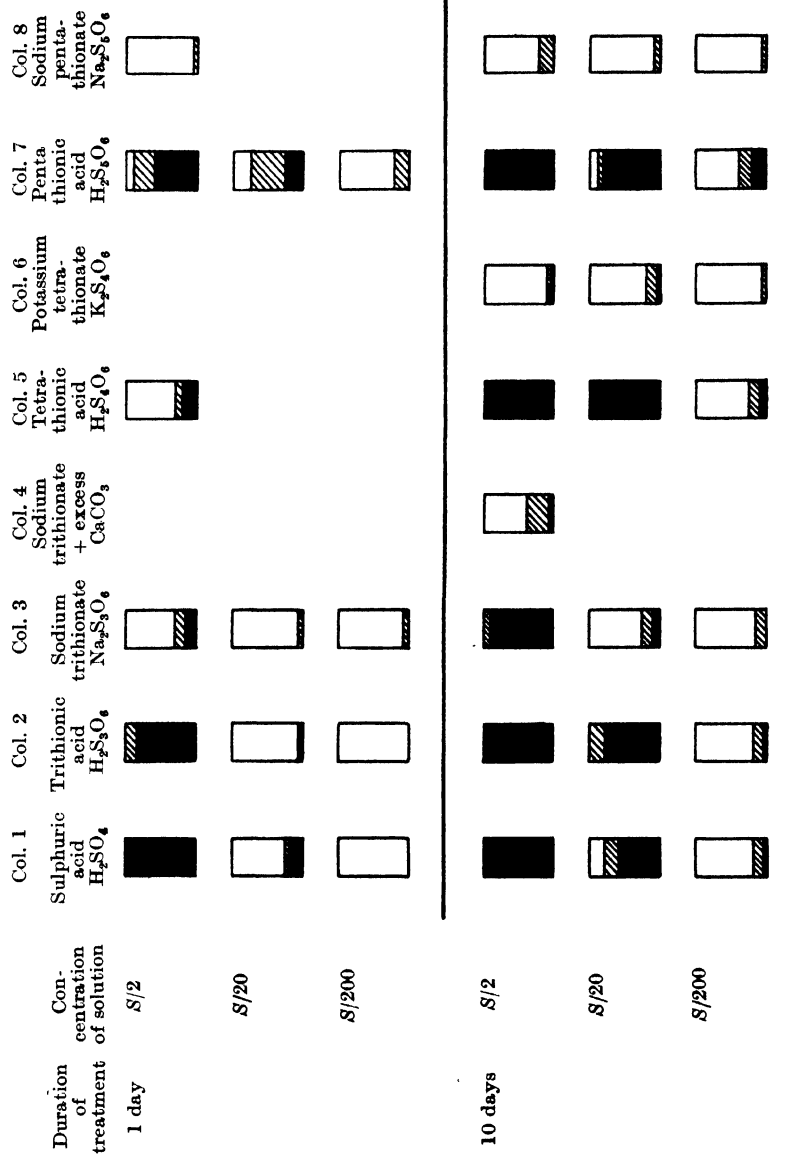


Fig. 4. Toxicities of polythionic acids and their salts compared with that of sulphuric acid.

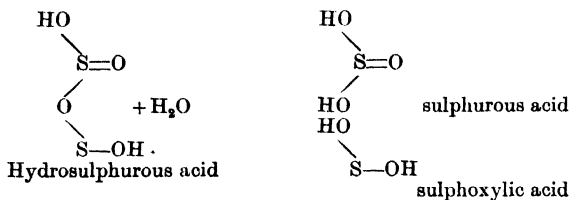
excess of calcium carbonate and by driving off sulphur dioxide by means of a slow stream of carbon dioxide (col. 4). The toxicity of the faintly acid solution will be referred to later (p. 182).

As none of the three polythionic acids is much more toxic than sulphuric acid, they do not appear to play an important part in the fungicidal action of sulphur towards *Synchytrium endobioticum*.

*Thiosulphuric Acid, Thiosulphate, etc.*

Sodium thiosulphate itself has no appreciable toxicity (Fig. 5, col. 3); but when it is acidified with sulphuric acid it has a high toxicity which shows itself with unusual rapidity, as indicated by the results obtained after treatment for one day only (Fig. 5, col. 2). Thiosulphuric acid is unstable except in dilute solution, as is shown by the fact that both the *S*/2 and the *S*/20 solutions rapidly deposit sulphur and give off sulphur dioxide. The toxicity of the solution might therefore be due either to the products of decomposition or to the undecomposed thiosulphuric acid. Sulphurous acid and polythionic acids, which are known to be products of the decomposition, are insufficiently toxic (Fig. 3, col. 2, Fig. 4, cols. 2, 5, 7) to account for the high toxicity of acidified thiosulphate solution.

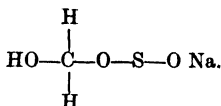
As acidified thiosulphate solutions have powerful reducing properties, toxicity tests were carried out with sodium hydrosulphite  $\text{Na}_2\text{S}_2\text{O}_4$ , also a powerful reducing agent, which may possibly be formed in the decomposition of thiosulphuric acid. These were carried out both in neutral and in acid solutions. The solution of sodium hydrosulphite, which gave at first a slight but increasingly acid reaction and smelt strongly of sulphur dioxide, was definitely toxic (Fig. 5, col. 5). A neutral solution of the salt was obtained by adding calcium carbonate and passing a stream of carbon dioxide through the solution to remove the sulphur dioxide formed. Under these conditions the toxicity was negligible (Fig. 5, col. 6). When sulphuric acid was added to sodium hydrosulphite the resulting solution showed the same high order of toxicity as acidified thiosulphate (Fig. 5, col. 4). Now hydrosulphurous acid  $\text{H}_2\text{S}_2\text{O}_4$  is a mixed anhydride of sulphurous and sulphylic acids; as may be seen from the following formulae:



Duration of treatment	Con- centration of solution									
		Col. 1 Sulphuric acid $H_2SO_4$	Col. 2 Acidified sodium thio- sulphate	Col. 3 Sodium thio- sulphate $Na_2S_2O_3$	Col. 4 Acidified sodium hydro- sulphite $[H_2S_2O_4]$	Col. 5 Sodium hydro- sulphite $Na_2S_2O_4$	Col. 6 Sodium hydro- sulphite neutral $Na_2S_2O_4$	Col. 7 Sodium formal- sulpho- xylate $NaCH_2SO_3$	Col. 8 Formal- dehyde $CH_2O$	Col. 9 Acidified sodium formal- dehyde sulpho- xylate
1 day	S/2									
	S/20									
	S/200									
10 days	S/2									
	S/20									
	S/200									

Fig. 5.

In solution it behaves as a mixture of these two acids. It has been shown that sulphurous acid (Fig. 3, col. 2) is not highly toxic, so sulphylic acid remains as the possible toxic agent. Neither free sulphylic acid nor its sodium salt are known to exist but the sodium salt is known in combination with formaldehyde as the compound sodium formaldehyde sulphyxylate.



This substance was found to be non-toxic in neutral solution (Fig. 5, col. 7). (The fact that it is less toxic than the formaldehyde which it "contains" is probably due to the formaldehyde suffering a molecular rearrangement on combination with the sodium sulphyxylate. That such a rearrangement does take place has been established by chemical means.) In acid solution (Fig. 5, col. 9) however it develops approximately the same degree of toxicity as acidified thiosulphate. Thus the three very unstable solutions obtained by liberating thiosulphuric, hydrosulphurous and sulphylic acids respectively from their salts by the addition of sulphuric acid are of the same high order of toxicity. From the work of Bassett and Durrant(2) and others cited by them it is obvious that these three acids are very closely interrelated, so that it seems possible that the toxicity of all three solutions may be due to the same substance formed from all three acids. All three solutions contain a variety of compounds but they are definitely known to contain thiosulphuric acid. It is possible therefore that the toxicity of all three solutions may be due to free thiosulphuric acid or some compound closely related to it, such, for instance, as Bassett and Durrant's postulated anhydro-acid.

An attempt was made to discover whether the toxicity of acidified thiosulphate solutions is due to some transitory compound formed as an intermediate product in the decomposition of the liberated thiosulphuric acid or to some compound contained in the more or less balanced solution which is known to be obtained a few hours after the acidification. The instability of many of the sulphur compounds formed and the length of exposure of the sporangia necessary for any measurable toxicity to show itself, constitute serious difficulties in determining the toxicities at all accurately. The following experiments, however, were carried out: a stock solution of *H*/10, *S*/20 acidified thiosulphate was made up and its toxicity was tested periodically. Samples of the clear

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liquid, as free from precipitated sulphur as possible<sup>1</sup>, were withdrawn at the end of 0, 1, 2, 4, 6, 8 hours, 1, 2, 4 and 7 days respectively. Sporangia were then treated with each sample for 24 hours. The toxicity figures obtained were constant within the limits of experimental error. Samples of an *H*/100, *S*/200 thiosulphuric acid solution were tested immediately after the solution was made up, at the end of 1 day and at the end of 7 days. The toxicity of all three samples was approximately the same.

If the toxicity is due to a transitory intermediate compound it should decrease after the disappearance of the compound sometime after the solution is made up. Since the toxicity does not decrease appreciably in 7 days it cannot be due to such a transitory intermediate compound but to a constituent of the balanced solution.

To determine which constituent of the balanced solution is responsible for the toxicity is of even greater difficulty and uncertainty. The chemical work done on acidified thiosulphate by Bassett and Durrant(2) and others, taken in conjunction with the facts already recorded in this paper, point to thiosulphuric acid itself or some compound closely related to it as the most likely toxic substance of those known to be present in the solution, viz. thiosulphuric acid (in small quantity), tri-, tetra-, and penta-thionic acids, sulphurous acid, sulphuric acid, sulphur (except in dilute solutions), etc. A further test was devised to give evidence on this question.

Since sulphurous acid is a decomposition product of thiosulphuric acid, the replacement of sulphuric acid by sulphurous acid in making up the solution will tend to produce a greater concentration of thiosulphuric acid in the equilibrium mixture obtained without appreciably affecting its acidity. A comparison of the toxicities of corresponding members of the two series was therefore made. (For details *re* making of these solutions see Appendix II, p. 189.)

As sulphuric and sulphurous acids in equal concentrations have about the same toxicity, any difference in toxicity found between members of a pair in the two series may be attributed to some indirect effect on the equilibrium mixture. The results (Fig. 6) show on the whole a slightly greater toxicity in the solutions in which sulphurous acid is in excess, but the differences are too small and irregular to warrant a definite conclusion.

<sup>1</sup> The liquid was not filtered because in doing so sulphur dioxide would be lost by evaporation and oxygen would be absorbed; both of these changes are likely to cause changes in the amounts of the other compounds in the balanced solution.

The precipitation of sulphur in the solutions after they had been allowed to stand overnight, *i.e.* 12 hours, is indicated in Fig. 6. An *S*/500 "excess sulphuric acid" solution, which was very slightly cloudy, corresponded with an "excess sulphurous acid" solution of concentration between *S*/200 (clear) and *S*/100 (cloudy), so that if the toxicity were due








Con- centration of solution	Amount sulphur pre- cipitated	Ap- pearance of solution	Excess sul- phuric acid	Excess sul- phurous acid	Amount sulphur pre- cipitated	Ap- pearance of solution
<i>S</i> /20	much				little	
<i>S</i> /50	little	cloudy			none	cloudy
<i>S</i> /100	none	cloudy			none	cloudy
<i>S</i> /200	none	cloudy			none	clear
<i>S</i> /500	none	very slightly cloudy			none	clear
<i>S</i> /1000	none	clear			none	clear
<i>S</i> /2000	none	clear			none	clear

Fig. 6.

to the separated sulphur then the excess sulphurous acid solutions should be between two and a half and five times as toxic as the excess sulphuric acid. The fact that the toxicities do not correspond with the degree of separation of sulphur suggests that there is no connection between toxicity and colloidal sulphur or sulphur in a finely divided state.

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Estimations of the amounts of thiosulphuric acid in the various solutions hitherto have given unreliable results so that it has not yet been possible to establish a quantitative relationship between thiosulphuric acid and toxicity. The qualitative evidence obtained, however, appears to justify the tentative conclusion that in solutions of the sulphur compounds considered, which are more toxic than sulphuric acid at the same hydrogen ion concentration, the excess toxicity is due to thiosulphuric acid, or some compound closely related to it, and formed from it on acidification.

On this theory the previously unexplained toxicity of trithionate solution becomes clear (pp. 176, 177). It becomes acid on standing, and in slightly acid solution it is known to decompose, giving rise to a certain amount of thiosulphuric acid. As the salt is non-toxic when its solution is kept neutral it seems probable that the toxicity of the solution of the salt which is not kept neutral is due to the thiosulphuric acid which is produced. Whereas both  $S/2$  and  $S/20$  solutions of sodium trithionate decolorised definite amounts of iodine after standing 10 days, solutions of trithionic acid, which was not more toxic than sulphuric acid, decolorised no iodine, showing that no thiosulphuric acid had been formed. Solutions of dithionic, tetrathionic and pentathionic acids and of their sodium salts also had no definite iodine value after standing 10 days.

Hence all the facts so far considered support the conclusions: first that the toxicities of all the sulphur acids so far considered which do not give thiosulphuric acid as a decomposition product are conditioned by their hydrogen ion concentrations; and secondly, when thiosulphuric acid is formed it bestows on the solution a greatly enhanced toxicity.

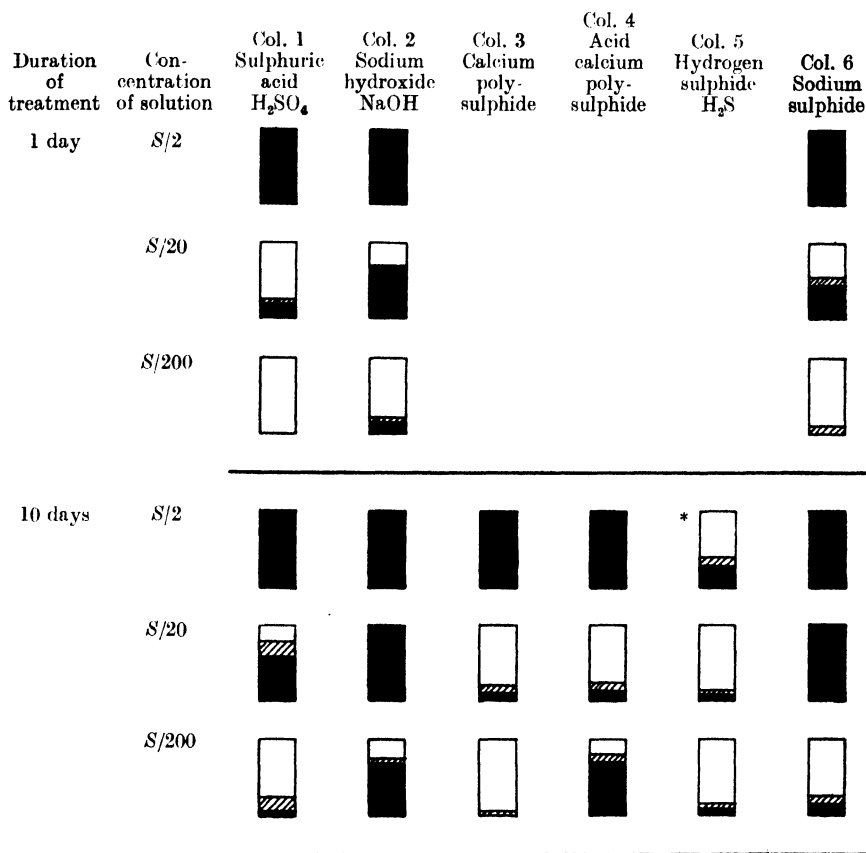
### *Sulphides and Polysulphides.*

Sulphides and polysulphides cannot be looked upon as intermediate products in the formation of sulphuric acid from sulphur, but they are included because of the possibility of their formation either from sulphur itself or from the intermediate products so far considered.

Though sulphur does not appear to exist in a normal soil in the state of sulphide, sulphuretted hydrogen is so often a product of decomposition of most of the compounds so far investigated that it seemed necessary to test the toxicity of sulphur in this form.

*Sulphuretted hydrogen.* To make up each solution the appropriate quantities of pure sodium sulphide and sulphuric acid were shaken together until all the solid had dissolved. An  $S/2$  solution could not be made up because sulphuretted hydrogen is insufficiently soluble, its

saturated solution at room temperature being approximately  $S/8$ . It is seen that sulphuretted hydrogen solution, or hydrosulphuric acid as this solution is sometimes called, has only a low degree of toxicity (Fig. 7, col. 5).



\* Saturated solution.

Fig. 7. Toxicity of sulphides, polysulphides, etc., compared with that of sulphuric acid.

*Sodium sulphide.*  $S/2$  sodium sulphide solution was prepared by dissolving the pure solid in water. The toxicity of the solution is probably explicable in terms of its alkalinity (Fig. 7, col. 6). *Sodium hydroxide* is seen to be more toxic than sulphuric acid when compared at equivalent concentrations.

*Calcium polysulphide* was prepared by the usual laboratory method. It is seen to be only slightly toxic (Fig. 7, col. 3) and such toxicity as it has may well be due to its alkalinity. In the "acid calcium polysulphide"

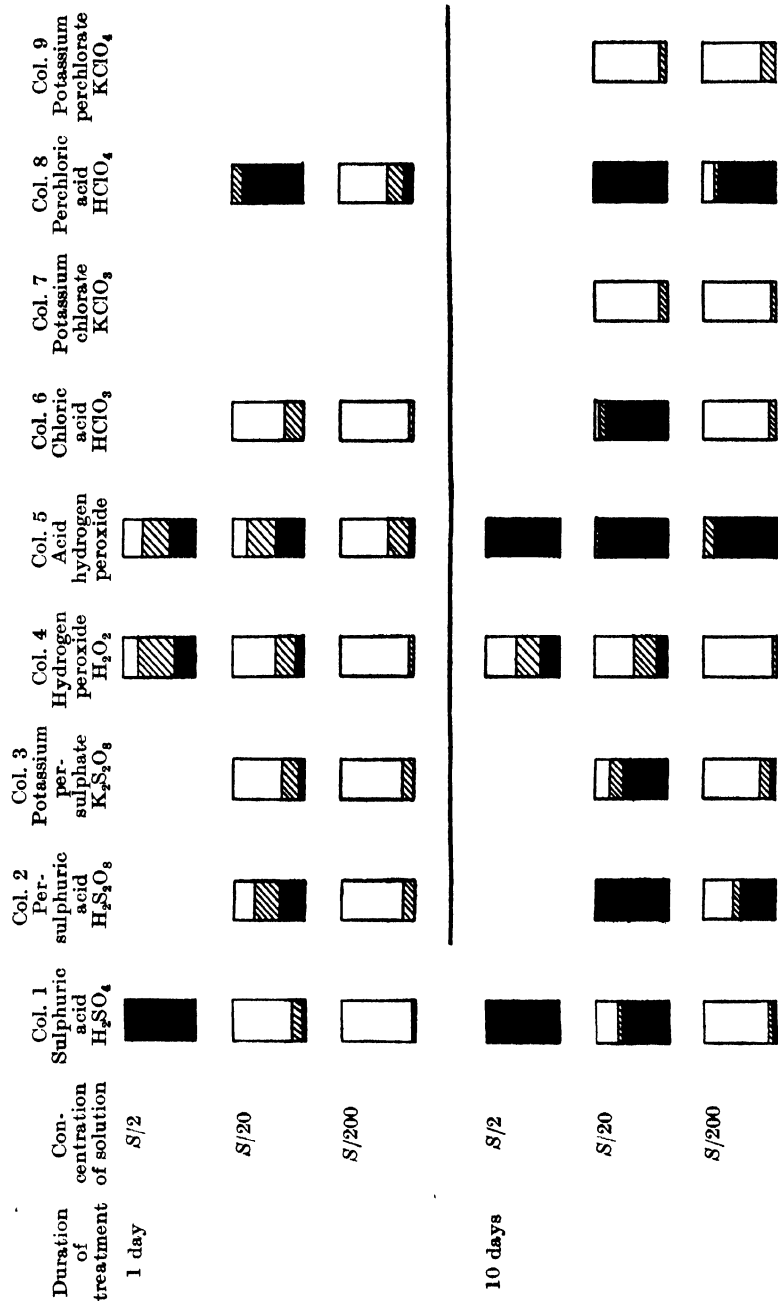


Fig. 8. Toxicity of oxidising agents.

the solutions were diluted with *N*/100 sulphuric acid instead of with water. The two more concentrated solutions still remained alkaline and their toxicities were little, if at all, affected by the addition of the small quantity of acid. The most dilute solution, however, was slightly acid and its toxicity was considerably increased by the addition of an amount of acid which alone had little toxicity (Fig. 7, cols. 3-4). The small quantity of thiosulphate which calcium polysulphide solutions almost invariably contain, which when acidified would set free thiosulphuric acid, was probably the cause of this increase in toxicity.

Sulphides and polysulphides do not appear to be sufficiently toxic to *Synchytrium endobioticum* to play any important part in the fungicidal action of sulphur, especially as not more than minute quantities of them ever occur in normal soils to which sulphur has been added; they were therefore not tested further.

#### *Oxidising Agents.*

It is known that the slow combustion of sulphur in the air gives rise to the formation of hydrogen peroxide. This in the presence of the sulphuric acid which also is a product of the slow oxidation of sulphur in air might well form persulphuric acid. In fact Barker, Gimingham and Wiltshire<sup>(1)</sup> observed that a potassium iodide starch paper held near warm moist sulphur soon became blue; a similar paper also turned blue when moistened with the liquid draining from the moist sulphur. The toxicities of hydrogen peroxide and persulphuric acid are therefore of interest although we have at present no evidence of the formation of either of these compounds in soil and sulphur mixtures.

*Persulphuric acid and potassium persulphate.* From Fig. 8, col. 2, it is seen that persulphuric acid has a high toxicity. After 10 days an *S*/200 solution, *i.e.* one to which 0.0016 per cent. sulphur was added in the form of persulphuric acid, was about as toxic as an *S*/20 solution of sulphuric acid, *i.e.* persulphuric acid is about ten times as toxic as sulphuric acid. Potassium persulphate solution also had a definite toxicity (Fig. 8, col. 3), which however was only about one-tenth that of the acid itself. By the end of the test the solution had become acid, so the compound was tested again in a solution kept neutral by being agitated gently with an excess of barium carbonate. Under these conditions its toxicity was small.

*Hydrogen peroxide* solutions of the same oxidising powers as the persulphate solutions were tested. Hydrogen peroxide in neutral solution was of low toxicity. In sulphuric acid solution it was highly toxic,

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in all probability, owing to the formation of persulphuric acid (Fig. 8, cols. 4 and 5).

*Chloric and perchloric acids* were tested to see whether other strong oxidising agents were also toxic. Chloric acid was of approximately the same toxicity as sulphuric acid and perchloric acid about ten times as toxic and the neutral salts non-toxic (Fig. 8, cols. 6, 7, 8, 9). To follow the question further was considered outside the field of the present investigation though interesting problems suggest themselves.

### SUMMARY.

The toxicities towards the winter sporangia of *Synchytrium endobioticum* of certain of the simpler sulphur compounds which are at all likely to be formed when sulphur is added to soil were tested and compared with that of sulphuric acid.

Sulphuric ( $\text{H}_2\text{SO}_4$ ), sulphurous ( $\text{H}_2\text{SO}_3$ ), dithionic ( $\text{H}_2\text{S}_2\text{O}_6$ ), trithionic ( $\text{H}_2\text{S}_3\text{O}_6$ ), tetrathionic ( $\text{H}_2\text{S}_4\text{O}_6$ ), and pentathionic ( $\text{H}_2\text{S}_5\text{O}_6$ ) acids were toxic and this toxicity was of the same order in each case at the same hydrogen ion concentration. Their neutral salts were non-toxic. These facts suggest that the toxicities of these acids are mainly due to their hydrogen ion concentrations.

Acidified solutions of sodium thiosulphate  $\text{Na}_2\text{S}_2\text{O}_3$ , sodium hydro-sulphite  $\text{Na}_2\text{S}_2\text{O}_4$  and sodium formaldehyde sulfoxylate were about ten times as toxic as sulphuric acid.

Evidence is brought forward which suggests that the toxicity of these acidified solutions, in excess of that accounted for by the hydrogen ion concentration, is due to the thiosulphuric acid present in each of them. In view of the experimental difficulties due to the instability of some of the compounds and the length of time taken by them to exert their toxic action on the fungus investigated, this conclusion must be regarded as tentative.

Of the other compounds tested sodium hydroxide was found to be a little more toxic than sulphuric acid and persulphuric acid about ten times as toxic; hydrogen peroxide, calcium polysulphide and sulphur-etched hydrogen were only slightly toxic.

## APPENDIX I.

NOTES ON THE PREPARATION, PURIFICATION AND ANALYSIS  
OF THE POLYTHIONATES.

The work described in the main part of the paper has been so dependent on the purity of certain of the compounds, especially the polythionates, that it seems desirable to append brief descriptions of the methods of preparation and purification of these compounds and analytical data establishing their degree of purity.

*Sodium trithionate*  $\text{Na}_2\text{S}_3\text{O}_6$  was prepared by the method described by Plessy (8). It was purified until free from thiosulphate. The crystalline precipitate was dried over quick-lime *in vacuo* and kept in a bottle with a well ground-in stopper.

*Analysis* (11. xi. 26). The chlorine value determined in a Bunsen<sup>1</sup> oxidation apparatus was 94.7 per cent. of the value calculated for pure  $\text{Na}_2\text{S}_3\text{O}_6$ . 0.5 gm. substance dissolved in water decolorised 1 drop of *N*/20 iodine but not 2 drops; hence no more than a trace of sulphite or thiosulphate was present. A solution of the substance gave a precipitate with barium chloride. 0.5 gm. substance was dissolved in water and the sulphate precipitated by means of barium chloride in the cold. The precipitate was spun down on the centrifuge, the supernatant fluid being discarded. The precipitate was alternately suspended in distilled water and spun down until the discarded supernatant fluid no longer gave a precipitate with silver nitrate. The precipitate was then washed into a weighed crucible, dried, ignited and weighed, its weight being 0.0478 gm., which corresponds to 0.0291 gm.  $\text{Na}_2\text{SO}_4$  or 5.8 per cent.  $\text{Na}_2\text{SO}_4$  in the salt. This figure is likely to be in excess of the true one because of the known property of barium sulphate precipitates of taking down salts with them, especially in the cold; thus the figure is in sufficiently close agreement with the one determined by difference, *i.e.*  $100 - 94.7 = 5.3$  per cent.

As an additional check the total sulphur in 0.5 gm. substance was determined by oxidation to sulphate and weighing as barium sulphate. Its weight was 1.4184 gm. Subtracting 0.0478 gm. *i.e.* the weight of  $\text{BaSO}_4$  equivalent to 0.0291 gm.  $\text{Na}_2\text{SO}_4$  we have left 1.3706 gm.  $\text{BaSO}_4$  which corresponds to 0.4667 gm.  $\text{Na}_2\text{S}_3\text{O}_6$  in the 0.5 gm. substance, *i.e.* 93.3 per cent. This figure is lower than the one calculated from the chlorine value, *viz.* 94.7; this fact no doubt is partially explained by the high value for the estimated  $\text{Na}_2\text{SO}_4$  content.

The analysis of the sample was taken to be

$\text{Na}_2\text{S}_3\text{O}_6$	94.7 per cent.	{	$\text{Na}_2\text{S}_3\text{O}_6$	95 per cent.
$\text{Na}_2\text{SO}_4$	5.3 „		$\text{Na}_2\text{SO}_4$	5 „
$\text{Na}_2\text{SO}_3 + \text{Na}_2\text{S}_2\text{O}_3$	trace only		$^{\circ}\text{SO}_3 + ^{\circ}\text{S}_2\text{O}_3$	trace only.

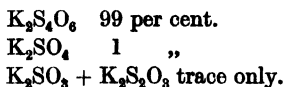
To make up the solution of trithionic acid the requisite amount of  $\text{Na}_2\text{S}_3\text{O}_6$  was added to sulphuric acid of the correct concentration. It had been ascertained that an excess of  $\text{Na}_2\text{SO}_4$  had no effect on the toxicity of  $\text{H}_2\text{SO}_4$ , and therefore presumably it would have none on that of  $\text{H}_2\text{S}_3\text{O}_6$  either.

*Potassium tetrathionate*  $\text{K}_2\text{S}_4\text{O}_6$  was prepared by the method described by F. Raschig (Schwefel- und Stickstoffstudien (1924), ch. xxiii).

<sup>1</sup> The newer and more accurate method due to Treadwell and Mayr (*Z. anorg. u. allg. Chem.* xii (1915), p. 127) had not at this time come to the notice of W. A. Roach who was responsible for the chemical part of this investigation.

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*Analysis* (29. x. 26). Similar methods to those employed for the trithionate gave the following figures:



*Barium pentathionate*  $\text{BaS}_5\text{O}_6$ . Though newer methods were tried the following method, which is but a slight modification of the original one for the preparation of pentathionates, gave the purest sample of pentathionate obtained in the present investigation.

A Wackenroder solution was made in the usual way by alternately passing sulphuretted hydrogen into a saturated solution of sulphur dioxide until there was no further smell of sulphur dioxide and allowing to stand overnight, then passing in sulphuretted hydrogen again on the morrow when the smell of sulphur dioxide had reappeared. In this way a solution containing a mixture of the polythionic acids is obtained, in addition to much precipitated sulphur. As more sulphuretted hydrogen is passed in so the proportion of pentathionic acid increases and that of the lower polythionic acids decreases; finally the pentathionic acid disappears with formation of more sulphur. At the third attempt the passing in of sulphuretted hydrogen was discontinued when most of the soluble sulphur was in the form of pentathionic acid. The solution was filtered and evaporated on a water bath to about half its bulk and filtered again to free from precipitated sulphur. To the almost clear solution barium carbonate was added with much stirring. Much sulphur dioxide was expelled, sulphur and barium sulphate were precipitated. When excess of barium carbonate had been added and well stirred in the liquid it was filtered again. A clear filtrate was obtained. To this filtrate first alcohol, then ether was added to precipitate the barium pentathionate, which was collected and washed with alcohol on a Buchner funnel and dried in a vacuum desiccator over quick-lime. A further crop of salt was obtained from the filtrate by saturation with calcium chloride and purifying the precipitate by taking up in water and precipitating with alcohol and ether.

The powder dissolved readily in water giving a clear solution. The solution gave a dense precipitate of sulphur on the addition of concentrated caustic soda. It also gave the other tests for pentathionate. A solution containing 0.1 gm. substance did not decolorise 1 drop of *N*/20 iodine. It was therefore practically free from sulphite and thiosulphate.

*Analysis.* 0.5 gm. powder was boiled with 50 c.c. *N*/10  $\text{KClO}_3$  + 25 c.c. conc.  $\text{HCl}$  and the precipitated  $\text{BaSO}_4$  filtered off, washed, ignited and weighed; 0.2656 gm.  $\text{BaSO}_4$  obtained, corresponding to the barium in the powder. The  $^{\circ}\text{SO}_4$  in the filtrate was precipitated with barium chloride in the usual way and weighed.

0.2656 gm.  $\text{BaSO}_4$  corresponding to Ba in powder.

1.3389 gm.  $\text{BaSO}_4$  corresponding to  $^{\circ}\text{SO}_4$  in powder.

$$\text{Ratio Ba : S : } : \frac{0.2656 \times \frac{137}{233}}{137} : 1.3389 \times \frac{32}{233} : : 1 : 5.041$$

Theory for  $\text{BaS}_5\text{O}_6$  1 : 5.000

$$1.3389 \text{ gm. } \text{BaSO}_4 = \frac{1.3389 \times 393}{233 \times 5} = 0.4517 \text{ gm. } \text{BaS}_5\text{O}_6$$

(Residue 0.0483 gm. water?  $\text{BaS}_5\text{O}_6$ , 2.3  $\text{H}_2\text{O}$ )

Powder was 90.34 per cent.  $\text{BaS}_5\text{O}_6$ .

The **chlorine** value was 91.3 per cent. of the value calculated for  $\text{BaS}_5\text{O}_6$ . The value 90.34 calculated from the barium sulphate precipitate was accepted as probably more accurate than the value 91.3 calculated from the chlorine value.

*S/2 solution.* Since 0.5 gm. powder gave 1.3389 gm.  $\text{BaSO}_4$  the powder contained  $\frac{1.3389 \times 100}{0.5} \times \frac{32}{233} = 36.78$  per cent. sulphur.

Therefore 25 c.c. *S/2* solution contain  $\frac{32}{2} \times \frac{25}{1000} = 0.4$  gm. sulphur. 0.4 gm. sulphur is contained in 1.088 gm. salt.

Therefore to make up 25 c.c.  $\text{Na}_2\text{S}_5\text{O}_6$  1.088 gm. of the above sample of  $\text{BaS}_5\text{O}_6$  were shaken with 25 c.c. *S/2*  $\text{Na}_2\text{SO}_4$  and the precipitated  $\text{BaSO}_4$  removed by centrifuging.

To prepare  $\text{H}_2\text{S}_5\text{O}_6$  *S/2*  $\text{H}_2\text{SO}_4$  was substituted for *S/2*  $\text{Na}_2\text{SO}_4$ .

## APPENDIX II.

NOTES ON THE PREPARATION OF THE SOLUTIONS REFERRED TO ON PAGES 180 AND 181.

A graduated series of pairs of solutions was made up, one member of each pair having its *pH* adjusted by means of an excess of sulphuric acid and the other member having an equivalent amount of sulphurous acid instead. Each pair differed from the preceding pair by being of half the concentration. 25.8 gm.  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  were dissolved in water and the solution made up to 2000 c.c.; thus it was an *S/10*  $\text{Na}_2\text{S}_2\text{O}_3$  solution. 1000 c.c. of this solution were set aside in a Winchester quart bottle; 800 c.c. of the remainder were made up to 2000 c.c. with water to form an *S/25*  $\text{Na}_2\text{S}_2\text{O}_3$  solution. 1000 c.c. of this solution were set aside in a Winchester quart bottle. In this way were prepared a litre each of solutions of  $\text{Na}_2\text{S}_2\text{O}_3$  of the following concentrations: *S/10*, *S/25*, *S/50*, *S/100*, *S/250*, *S/500*, *S/1000*, which were placed in a row in Winchester quart bottles. In a similar manner by starting with 24.8 gm.  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O} + 25.2$  gm.  $\text{Na}_2\text{SO}_3$  for the first solution were prepared a series of solutions of the following concentrations: *S/10*  $\text{Na}_2\text{S}_2\text{O}_3$ , *S/20*  $\text{Na}_2\text{SO}_3$ ; *S/25*  $\text{Na}_2\text{S}_2\text{O}_3$ , *S/50*  $\text{Na}_2\text{SO}_3$ ; ... *S/1000*  $\text{Na}_2\text{S}_2\text{O}_3$ , *S/2000*  $\text{Na}_2\text{SO}_3$ . These solutions also were contained in Winchester quart bottles and placed in a row. By the side of each of these rows of bottles were placed bottles containing 1000 c.c. of sulphuric acid of the following concentrations: *H/5*, *H/12.5*, *H/25*, *H/50*, *H/125*, *H/250*, *H/500*. Finally the appropriate acid solution was added to the " $\text{Na}_2\text{S}_2\text{O}_3$ " or " $\text{Na}_2\text{S}_2\text{O}_3 + \text{Na}_2\text{SO}_3$ " solution as rapidly as possible and mixed thoroughly. In this way were obtained two series of solutions each of which consisted of solutions of the following concentrations of  $\text{H}_2\text{S}_2\text{O}_3$ ; *S/20*, *S/50*, *S/100*, *S/200*, *S/500*, *S/1000*, *S/2000*. The acidity of these solutions in regard to  $\text{H}_2\text{S}_2\text{O}_3$  was *H/40*, *H/100*, ... *H/4000*. In the first series there was an excess of sulphuric acid to make up the total acidities to *H/10*, *H/25*, ... *H/1000*; in the second series the excess of acid was in the form of sulphurous acid. There was also more sodium sulphate in the second series which, however, can be neglected as far as its toxic effect is concerned.

## REFERENCES.

- (1) BARKER, B. T. P., GIMINGHAM, C. T., WILTSHIRE, S. P. (1919). Sulphur as a Fungicide. *Ann. Rpt. Long Ashton Res. Sta.* p. 57.
- (2) BASSETT, H. and DURRANT, R. G. (1927). The Inter-relationships of the Sulphur Acids. *Journ. Chem. Soc.* pp. 1401-68.
- (3) CROWTHER, E. M., GLYNNE, MARY D. and ROACH, W. A. (1927). Sulphur Treatment of Soil and the Control of Wart Disease of Potatoes in Pot Experiments. *Ann. App. Biol.* xiv, 422-7.
- (4) GLYNNE, MARY D. (1926). The Viability of the Winter Sporangium of *Synchytrium endobioticum* (Schilb.) Perc., the Organism causing Wart Disease in Potato. *Ibid.* xiii, 19-36.
- (5) GUITTONNEAU, G. (1925). Sur la formation d'hyposulfites aux dépends du soufre par les microorganismes du sol. *C.R. Acad. Sci. (Paris)*, CLXXX, 1142-4.
- (6) — (1926). Sur l'oxydation microbienne du soufre. *Ibid.* CLXXXII, 661-2.
- (7) GUITTONEAU, G. and KEILING, J. (1927). Sur la solubilisation du soufre élémentaire et la formation des hyposulfites dans une terre riche en azote organique. *Ibid.* CLXXXIV, 898-901.
- (8) PLESSY, M. (1844). Note sur la préparation du sulphyposulfate de potasse. *Annales de Chimie et de Physique*, III<sup>me</sup> Série, xi, 182-8.
- (9) ROACH, W. A., GLYNNE, MARY D., BRIERLEY, WM. B. and CROWTHER, E. M. (1925). Experiments on the Control of Wart Disease of Potatoes by Soil-Treatment with Particular Reference to the Use of Sulphur. *Ann. App. Biol.* xii, 152-190.
- (10) ROACH, W. A. and BRIERLEY, WM. B. (1926). Further Experiments on the Use of Sulphur in Relation to Wart Disease of Potatoes. *Ibid.* xiii, 301-307.
- (11) WEISS, FREEMAN (1925). The Conditions of Potato Wart. *Amer. Journ. Bot.* xii, 413-442.
- (12) YOUNG, H. C. (1922). The Toxic Property of Sulphur. *Ann. Miss. Bot. Gard.* ix, 403.

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## ADDITIONAL HOSTS OF *SYNCHYTRIUM* *ENDOBIOTICUM* (SCHILB.) PERC.

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(With Plates XVIII and XIX.)

### INTRODUCTION.

IN connection with the investigations on Wart Disease of Potato being carried out in the Rothamsted Experimental Station it was found necessary to test the infectivity of *Synchytrium endobioticum* on a number of additional species of plants. In selecting plants for this experiment, it was considered advisable to include not only such species of the Solanaceae as occur in the British flora and in cultivation in this country, but also certain plants which are weeds and crops of other countries in which *Synchytrium endobioticum* has been recorded.

Four species of American weeds were selected, *Solanum Commersonii* and *Solanum Jamesii* to represent the tuber bearing class; *Solanum nodiflorum* and *Nicandra physalodes* among the non-tuber bearing weeds. With the exception of *Solanum nodiflorum*, these species have been tested in America and *Solanum Commersonii*(7) and *Solanum Jamesii*(7) found susceptible to wart disease, while infection has not been recorded in *Nicandra physalodes*.

Among the British solanaceous weeds *Solanum nigrum*, *Solanum dulcamara*—including a white variety *Solanum dulcamara alba*—*Datura stramonium* and *Hyoscyamus niger* were chosen. Of these *Solanum nigrum*(2), *Solanum dulcamara*(2) and *Hyoscyamus niger*(3) have previously been found susceptible and were included rather in the nature of controls.

*Solanum dulcamara* var. *villosissimum* and *Solanum villosum* are common continental weeds. *Solanum villosum* is occasionally found in this country on waste ground in the neighbourhood of docks. *Petunia violacea*, *Petunia alba*, *Salpiglossis sinuata*, *Nicotiana affinis* and *Nicotiana Sanderae* are members of the Solanaceae which are commonly met in garden cultivation in this country. As examples of crop plants tobacco, tomato, egg plant and winter cherry were chosen. Of the above infection has been recorded in tomato(7) and suspected in *Salpiglossis*.

## CULTIVATION OF EXPERIMENTAL PLANTS.

The plants were all raised and grown in a warm glasshouse throughout the experiments. The seed was sown in clean soil, and as soon as the young plants were large enough to handle, they were transplanted into pots of seven inches diameter. The compost was made of fresh potting soil well mixed with one half its bulk of soil contaminated with *Synchytrium endobioticum*. Previous workers(4, 5), investigating the conditions for infection of the potato by *Synchytrium*, have found that a certain period of excessive moisture in the soil results in a greater percentage of infection. In order to maintain these conditions, the pots were not crocked in the usual manner but cavings or sphagnum moss was used instead, so that there might be a free upward passage of water from the saucers in which the pots stood. The seedlings were treated normally until they were well established, *i.e.* from seven to fourteen days after transplanting, then earthenware saucers were placed under the pots and the amount of water supplied was increased so that some water always remained in the saucers.

Although the plants were grown under these conditions of practically water-logged soil until the experiments were concluded they grew well, with very few exceptions, and looked particularly healthy—flowering and fruiting as satisfactorily as under more normal conditions.

## METHODS OF INOCULATION.

(a) *Soil inoculation.*

Decaying potato warts were finely ground and mixed with the compost. All the plants were grown in this contaminated soil which was kept very moist to give the most suitable conditions for the germination of the contained winter sporangia. In susceptible potatoes rapid and profuse infection is usually obtained in the collar region. A small amount of very highly contaminated soil (ground wart mixed with a little sand) was therefore placed around the collars of the experimental plants to increase the chance of infection in that area.

(b) *Glynné's "green wart" method.*

This method(5) depends on the more rapid and certain germination of the summer sporangia in a green wart in comparison with the resting sporangia contained in a black wart. The method consists of placing a piece of young and freshly formed wart in contact with the plant to be

inoculated. A film of water is maintained between the plant and the wart to ensure the correct conditions for infection, *i.e.* free water into which the zoospores from the summer sori may be discharged.

All the wart employed in these experiments was derived from Arran Chief potatoes. This variety is very susceptible to *Synchytrium endobioticum* and the warts are produced rapidly and in abundance on the young shoots. The warts were removed as soon as they were of a convenient size to handle, from 0.5 cm. in diameter upwards, and while of a pale yellow or green colour.

The actual procedure was as follows. A leaf axil on a plant was selected in which the young axillary shoot was just beginning to grow; about the third node from the ground level in most cases. A small wart or piece of wart was placed in contact with the young shoot and base of the leaf and then covered with moist sphagnum moss. The wart and moss were tied firmly in position with bast to ensure contact with the host plant. The sphagnum was moistened at regular intervals with water to keep the wart fresh and to preserve a film of water between the wart and young shoot.

In *Hyoecyamus niger* the wart was tied to one of the young leaves as the plants were in the sessile rosette form, not having produced a central stem.

The wart was left in contact with the shoot for varying periods and the plants were examined at intervals for signs of infection. The usual period of contact was three weeks, although certain plants showed warted outgrowths after fifteen days.

*(c) Attempted localisation of infection by swarm spores.*

This method is a modification of Glynne's method and was tested on Arran Chief potatoes. The aim was so to localise the point of inoculation that the manifestation of the disease might be confidently looked for in a small circumscribed area, thus saving much tedious examination by lens and eye over a comparatively large area of the shoot.

Tubers were selected with a shoot about 1 cm. long. The latter was preferably situated at the rose end and developing somewhat obliquely outwards from the long axis of the tuber. The other shoots were rubbed off. Small glass cups were made with a minute aperture near the base and so placed that the aperture came in contact with the shoot. Each cup was held in position against a young shoot by brass wire. A small piece of green wart was put in the cup and a drop of water added to

ensure contact between the wart and the small area of tissue exposed through the aperture in the bottom of the cup.

The tubers were placed in an incubator in wire cages and the wart frequently moistened. The shoots were examined at intervals for infection by *Synchytrium* but in no case was infection obtained. Although the method was not successful in these tests there seems reason to consider that some slight modification in the technique might make this an extremely useful method.

#### EXAMINATION OF PLANTS.

##### *Infection from the soil.*

The plants were removed from the pots and the roots and collar carefully washed free of soil. They were then thoroughly examined and any suspected portions removed and hand sectioned.

##### *Infection by green wart method.*

The young shoot and leaves of the plant which had been in contact with the wart were minutely examined and any portions bearing the slightest visible excrescences were removed and fixed in Flemming's weak solution. In some cases, even where no roughness or blistering could be discerned, samples were removed and fixed, in the hope of finding the parasite present in the tissues without any outward manifestation of its presence. The material was microtomed and permanent preparations made. The results of the experiments are given in Tables I-III.

#### RESULTS.

These experiments on the testing of Solanaceous plants for susceptibility to *Synchytrium endobioticum* have resulted in the finding of several new hosts for this fungus. Previous work on the infectivity of *Solanum nigrum*, *Solanum dulcamara* and *Lycopersicum esculentum* has been confirmed, and the following new hosts are here recorded: *Solanum dulcamara* var. *villosissimum*, *Nicandra physalodes*, *Solanum dulcamara alba*, *Solanum nodiflorum* and *Solanum villosum*. Infection has not been obtained in *Datura stramonium*, *Hyoscyamus niger*, *Nicotiana affinis*, *Nicotiana Sanderae*, *Nicotiana Tabacum*, *Petunia violacea*, *Petunia alba*, *Physalis Francheti*, *Salpiglossis sinuata*, *Solanum Commersonii*, *Solanum Jamesii* and *Solanum melongena esculentum*.

The reaction to the *Synchytrium* varied widely in the different hosts. On the majority of the species a definite warty outgrowth developed.

The production of a wart however was not taken as a criterion for infection. The presence of the parasite, in any stage in the tissues of the plant, has been accepted as evidence of a positive infection of the plant by *Synchytrium*, and the species so infected has been recorded as susceptible to the disease. This was considered the best attitude to adopt with regard to the very slight infections noted on some of the species, for example *Solanum dulcamara* var. *villosissimum*, *Nicandra physalodes* and *Solanum dulcamara alba*, because, although the infection was so slight, the parasite appeared to be perfectly healthy and capable of further development. The *Synchytrium* was never observed to be in process of disintegration after its entry into the host cell had been effected (1). If the host reaction or the amount of outgrowth developed is taken as an indication of the susceptibility of the host (6), then tomato, which produces warts easily, can be regarded as the most susceptible species examined. With this host a varietal test was conducted, the "green wart" method being employed for inoculation purposes, and of the fourteen varieties tested, all with the exception of three, Buckley, Sutton's Every Day and Sutton's Maincrop, proved susceptible to *Synchytrium* (see Table II). Kondine Red produced the most rapid reaction to the infection, the warted areas on the leaves being from 1.0-1.5 cm. in length within fourteen days of inoculation. Little pegs of tissue of about 0.5 mm. in diameter and 1-2 mm. in height were observed on the leaflets of the variety of "Fillbasket." These occurred either singly or in groups of two or three. Microscopic examination showed them to contain the fungus in various stages of growth. This form of host reaction is presumably an early stage before the distortion becomes more general over the leaf. In most cases the leaves of the plants of the successfully inoculated varieties were visibly distorted by the development of the fungus. The test confirms the fact that susceptibility to the disease is widespread among the varieties of tomato commonly used in cultivation in this country.

The *Solanum* group can be classed next to the tomato in degree of susceptibility or amount of host reaction. In this group warts were not produced with the frequency and regularity shown by the tomato. A definite warted outgrowth was produced on the leaf in one plant of *Solanum nigrum*, *Solanum nodiflorum* and *Solanum villosum* respectively. The remainder of the infections in these three species and in *Solanum dulcamara alba* were not easily visible as the hypertrophy of the leaf tissues had only just begun.

In the remaining hosts, *Solanum dulcamara* var. *villosissimum* and *Nicandra physalodes*, the infections were not visible to the naked eye.

The leaf of the former, which was found to contain *Synchytrium* in various stages, showed no visible external symptoms but in microtome section appeared to be slightly thicker than the normal leaf. There was none of the usual distortion which accompanies infection as in the tomato.

The infection in *Nicandra physalodes* was extremely slight. A young parasite was discovered near the median vein of a young leaf which was still folded, but its detection was quite impossible without microscopic examination. The pad of cells in which the parasite was situated is normal tissue covering the vein and not the beginning of hypertrophy of the host tissues.

The duration of these experiments was generally from fifteen to twenty-one days, and never more than five weeks, from the time the green wart was placed in contact with the shoot until the shoot was removed for examination. It is possible therefore, had it been practicable to leave the plants for a longer period, that the host reactions would have developed to a greater extent and that mature resting sporangia would have been found more abundantly in the tissues.

A varietal test was conducted with tobacco, six varieties being used (see Table III) and the green wart method employed as previously described. The results of the experiment were entirely negative.

The green wart test on *Solanum Commersonii* and *Solanum Jamesii* was attempted too late in the season, the haulms reaching maturity and withering before results could be expected. These plants will be tested again during the coming season and it is hoped to confirm Weiss's results.

In all experiments there was a total failure of any infection of the plants from the soil which indicates that for experimental purposes the green wart method of shoot inoculation is the more reliable. It is difficult to suggest any feasible explanation of the lack of infection from soil inoculation.

#### SUMMARY.

Infection of numerous species of Solanaceae by *Synchytrium endobioticum* has been obtained using Glynne's "green wart" method. Plants grown in contaminated soil did not show infection. The following new hosts are recorded: *Solanum dulcamara* var. *villosissimum*, *Nicandra physalodes*, *Solanum dulcamara alba*, *Solanum nodiflorum* and *Solanum villosum*.

In certain hosts the fungus may occur in the tissues with little or no external sign of its presence.

Table I.

*Infectibility of different species of host plant.*

Species	No. of plants inoculated	No. of plants infected	
		(1) Contaminated soil	(2) Green wart method
<i>Datura stramonium</i>	13	—	—
<i>Hyoscyamus niger</i>	4	—	—
<i>Solanum dulcamara</i> var. <i>villosissimum</i>	5	—	1
<i>Lycopersicum esculentum</i>	47	—	27
<i>Nicandra physalodes</i>	13	—	1
<i>Nicotiana affinis</i>	4	—	—
<i>Nicotiana Sanderae</i>	4	—	—
<i>Nicotiana Tabacum</i>	22	—	—
<i>Petunia violacea</i>	4	—	—
<i>Petunia alba</i>	4	—	—
<i>Physalis Francheti</i>	12	—	—
<i>Physalis</i> (Red fruit var.)	4	—	—
<i>Salpiglossis sinuata</i>	10	—	—
<i>Solanum Commersonii</i>	4	—	No test
<i>Solanum dulcamara</i>	2	—	1
<i>Solanum dulcamara alba</i>	4	—	1
<i>Solanum Jamesii</i>	4	—	No test
<i>Solanum melongena esculentum</i>	8	—	—
<i>Solanum nigrum</i>	4	—	2*
<i>Solanum nodiflorum</i>	8	—	4*
<i>Solanum villosum</i>	4	—	1*

\* One visible wart.

Table II.

*Tomata. Varietal test.*

Variety	No. of plants inoculated.	No of plants infected
	Green wart method	
A 1	3	1
Ailsa Craig	2	1
Best of All	3	3
Blaby	2	1
Buckley	2	—
Comet	2	1
Earliest of All	3	1
Fillbasket	2	1
Kondine Red	16	14
Manx Marvel	2	1
Princess of Wales	3	2
Sutton's Early Market	3	1
Sutton's Every Day	2	—
Sutton's Maincrop	2	—

Table III.

*Tobacco. Varietal test.*

Variety	No. of plants inoculated. Green wart method	No. of plants infected
Connecticut	3	—
Kentucky	3	—
Kentucky White Stem	3	—
Maryland	3	—
Virginia	3	—
White Burley	7	—

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## REFERENCES.

- (1) CARTWRIGHT, K. (1926). On the Nature of the Resistance of the Potato to Wart Disease. *Ann. of Bot.* XL, 158, 391.
- (2) COTTON, A. D. (1916). Host Plants of *Synchytrium endobioticum*. *Kew Bull. Misc. Inform. London*, x, 272-275.
- (3) ESMARCH, F. (1925). Nachtschattengewächse als Wirtspflanzen des Kartoffelkrebspilzes (*Synchytrium endobioticum*). *Angew. Bot.* VII, 2, 108-120.
- (4) ——— (1927). Untersuchungen zur Biologie des Kartoffelkrebses. II. *Ibid.* IX, 2, 88-124.
- (5) GLYNNE, M. D. (1925). Infection Experiments with Wart Disease of Potatoes (*Synchytrium endobioticum* (Schilb.) Perc.). *Ann. App. Biol.* XII, 1, 34-60.
- (6) KOHLER, E. (1924). Ueber die Beziehungen des Kartoffelkrebseserregers (*Synchytrium endobioticum* (Schilb.) Perc.) zu seiner Wirtspflanze. *Centralbl. für Bakt.* 2, LXI, 1-4, 32-37.
- (7) WEISS, F. (1925). The Conditions of Infection in Potato Wart. *Am. Journ. Bot.* XII, 7, 413-443.

## EXPLANATION OF PLATES XVIII AND XIX.

## PLATE XVIII.

- Fig. 1. Winter sporangium of *Synchytrium endobioticum* in leaf tissue of *Solanum nigrum*.  
 Fig. 2. Young winter sporangium in leaf tissue of *Solanum dulcamara*.  
 Fig. 3. Five summer sporangia in a sorus in warted leaf of Tomato, variety Manx Marvel.  
 Fig. 4. Young winter sporangium in warted leaf of Tomato, variety Best of All.  
 Fig. 5. Young sporangia in warted leaf of Tomato, variety Kondine Red.

## PLATE XIX.

- Fig. 1. Sporangia in various stages of development in leaf tissues of *Solanum nodiflorum*.  
 Fig. 2. Warted leaf of *Solanum nodiflorum* showing sporangia in various stages of development.  
 Fig. 3. Sporangium in leaf tissues of *Solanum dulcamara* var. *villosissimum*.  
 Fig. 4. Young sporangium in leaf tissues of *Nicandra physalodes*.  
 Fig. 5. Young sporangium in leaf of *Solanum dulcamara* *alba*.  
 Fig. 6. Resting sporangia in warted leaf of *Solanum villosum*.

Addendum. Since going to Press infection has been obtained on *Solanum Jamesii*.

(Received February 27th, 1929.)



Fig. 1.

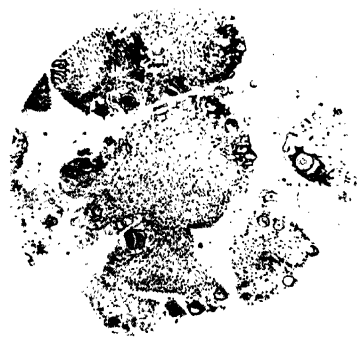


Fig. 2.



Fig. 3.



Fig. 4.



Fig. 5.



Fig. 6.





Fig. 1.

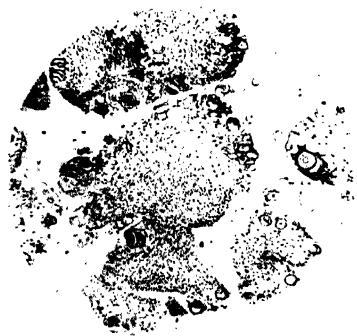


Fig. 2.

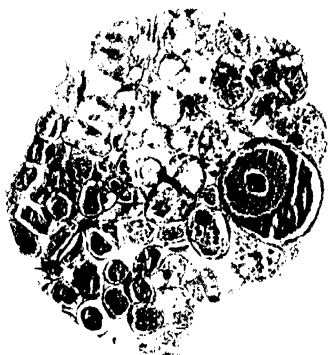


Fig. 3.



Fig. 4.



Fig. 5.



Fig. 6.



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# SULPHUR AS A SOIL FUNGICIDE AGAINST THE POTATO WART DISEASE ORGANISM.

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(With Eight Text-figures.)

## INTRODUCTION.

THE variability in the toxicity of sulphur, when used as a fungicide against the organism that causes the Wart Disease of Potatoes, has formed the subject of a number of earlier papers (2, 10, 11, 12) and led in 1926 to an investigation in the laboratory into the underlying causes. The acidity that results from the oxidation of the sulphur to sulphuric acid, a process which is known to complete itself in soil in a few weeks, having been found to be insufficient to account for the fungicidal action (2, 10, 12), the idea suggested itself that some other compound, formed as a result of the interaction of sulphur and soil, might be responsible. In assessing the importance of any such compound, account has to be taken both of its concentration and of its degree of toxicity. At the outset, therefore, the investigation proceeded along two lines: (1) the exploration of the chemical changes undergone by sulphur in soil, and (2) the determination of the toxicities of compounds formed or at all likely to be formed under these conditions. The second part of the problem has already been the subject of a communication (12).

The first-named part of the investigation, viz. the exploration of the chemical changes undergone by sulphur in soil, which forms the subject of this paper, proved much more difficult. Experiments of as simple a nature as possible were carried out to determine what kinds of compounds are formed when sulphur is incorporated with soil, and to get some idea of the conditions favouring the formation of any such compounds as happened also to be toxic to the Wart Disease fungus. These qualitative, or only partially quantitative, experiments were to have been preliminary to more carefully planned quantitative ones, but owing to the writer's transference to another sphere of work even the

<sup>1</sup> The work described on pp. 85-92 was carried out after the writer had taken up his new appointment.

preliminary experiments had to be somewhat curtailed. Nevertheless the results obtained, so far as they go, do seem to throw some light on the problem and do at least suggest a possible mode of action of sulphur as a soil fungicide, hence they are put on record here. Compounds which it seems at all likely would be formed as a result of the interaction between sulphur and soil fall conveniently into four main classes:

- (i) Sulphuretted hydrogen and other sulphides.
- (ii) Compounds intermediate in state of oxidation between sulphur and sulphuric acid.
- (iii) Sulphuric acid and sulphates.
- (iv) The persulphuric acids and their salts, together with hydrogen peroxide and ozone.

These four classes are readily distinguished chemically; sulphuric acid and sulphates alone give an immediate precipitate with barium chloride, whereas members of the first two classes give such a precipitate only after preliminary treatment with strong oxidising agents; members of the fourth class alone give a blue coloration with acidified potassium iodide and starch. The first step, therefore, was to seek evidence of the formation of any members of these classes of compounds under conditions as similar as possible to those in the field. The tests were carried out mainly on aqueous extracts of the soil.

#### EXPERIMENTAL METHOD.

*Soil.* Most of the experiments were carried out mainly with two kinds of soil:

1. *Ormskirk* soil, taken from the field at the Potato Testing Station of the National Institute of Agricultural Botany. This was a typical black, peaty, sandy potato soil.

2. *Rothamsted* soil, taken from the electro-culture enclosure. This soil was a stiff clay, containing numerous small pieces of calcium carbonate.

The soil in each case was allowed to dry under cover until it could readily be crumbled, it was then rubbed through a 2 mm. sieve. The portion passing through was thoroughly mixed and moistened with water by means of a spray until a handful on being compressed stuck together, but on gentle rubbing with the finger disintegrated again into its original particles. Plants grow readily in such a soil. After thorough mixing a quantity was weighed out and spread in a uniform layer of about  $\frac{1}{2}$ -inch thickness on a clean sheet of paper. The requisite quantity of finely ground and sifted sulphur was then distributed as uniformly

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as possible through a "100 mesh" sieve over the soil. After a preliminary mixing by hand the soil-sulphur mixture was transferred through a 2 mm. sieve to another piece of paper, care being taken that each charge of the sieve consisted of portions from all parts of the heap. This sifting and mixing process was repeated at least six times.

The prepared soil-sulphur mixture was usually weighed out in 100 gm. lots and put into glass test-tubes placed in constant temperature baths at 0°, 15° and 30° C. respectively, the ends of the tubes being either open to the air or lightly plugged with cotton wool. The moisture content was made up periodically by dropping water slowly from a pipette on to the mixture, care being taken not to cause the particles to run together.

*Methods of soil extraction.* Three methods of extraction of the soil-sulphur mixture were used.

1. The 100 gm. sample was placed in a glass tube provided at the lower end with a rubber stopper through which passed a No. L. 1 Chamberland filter candle. Water was added, the upper end of the tube then closed with a rubber stopper and the whole thoroughly shaken by hand. The candle was connected to a filter pump through a flask, and the upper rubber stopper removed. This method gave a small quantity of extract within a few minutes of the wetting of the mixture, and it was convenient for use when speed was essential. It was, however, inconvenient for quantitative work, because the rate of filtration slowed down as the pores of the candle became clogged, and unless some salt was added with the second lot of extracting water the clay became deflocculated and rendered a second filtration very slow indeed. (See Fig. 1.)

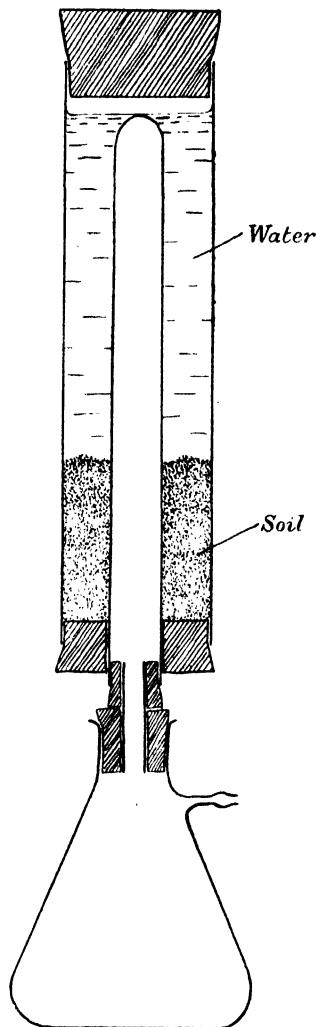


Fig. 1. Apparatus No. 1 for obtaining rapidly a small quantity of extract from soil.

2 (a). The 100 gm. sample of soil-sulphur mixture was placed in a Soxhlet extraction thimble standing in a glass funnel, and water was allowed to drop on it at such a rate that it moved from crumb to crumb by capillarity without the intervening air-spaces becoming water-logged. In this way the most concentrated soil extract was obtained, and it was perfectly clear. 5 c.c. of normal sulphuric acid added to and mixed with 100 gm. of the soil-sulphur mixture could be extracted completely in about 12 hours, as may be seen from the following figures which are represented graphically in Fig. 2.

	Volume in ml.	$\equiv$ ml. <i>N</i> /10 NaOH	Total sulphur extracted by 1 ml. water %
1st percolate	50	13.3	0.519
2nd "	70	24.3	0.678
3rd "	50	12.2	0.471
4th "	60	1.4	0.046
5th "	50	0.0	0.00
		<hr/> 51.2	

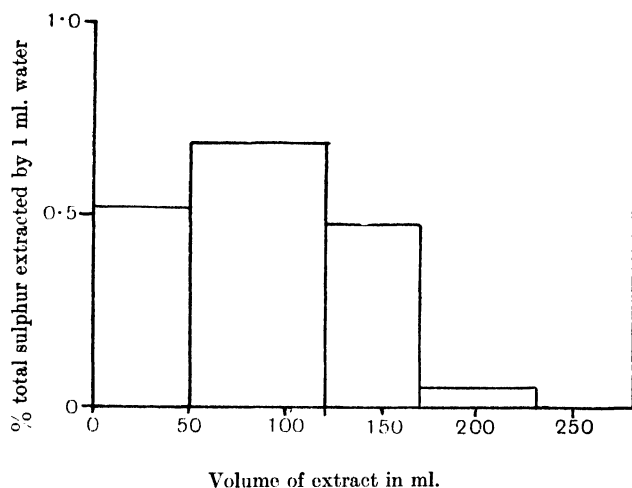


Fig. 2. Amounts of sulphur extracted per ml. water by successive portions of water.

Sulphuric acid added  $\equiv$  51.25 ml. *N*/10 NaOH. Hence 230 ml. water extracted all the sulphuric acid from 100 gm. soil.

2 (b). For extracting larger quantities use was made of an elongated Büchner funnel, the elongation consisting of a glass tube of the same diameter as the funnel and fixed thereto by means of surgical rubber tape.

3. The apparatus shown in Fig. 4 was used to obtain extracts under rather less aërobic conditions than in the last method.

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### *Estimation of sulphur in soil.*

*Extraction.* Acetone was found unsatisfactory as a solvent, because it extracted from soil a dark gummy substance which, on subsequent

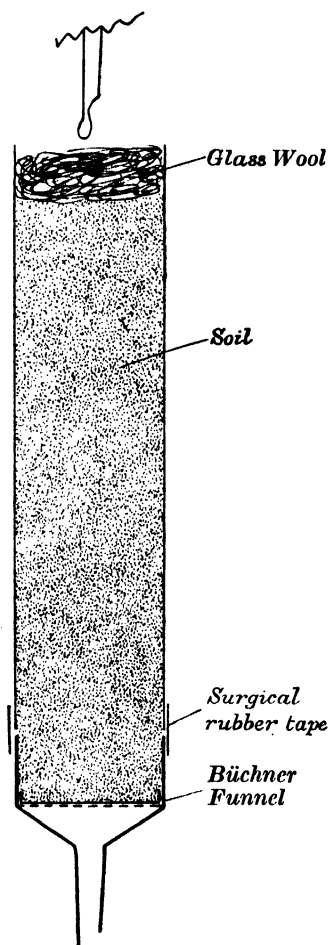


Fig. 3. Apparatus No. 2 *b* for extracting larger quantities of soil.

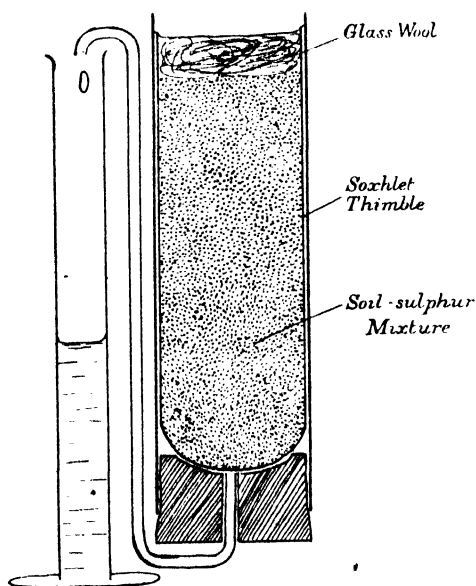


Fig. 4. Apparatus No. 3 for obtaining soil extract under partially aerobic conditions.

oxidation of the extracted sulphur with bromine and nitric acid or by other means, gave a yellowish resinous substance highly resistant to oxidation. Carbon tetrachloride, however, extracted the sulphur almost free from the contaminating gummy substance, even from Ormskirk soil, which was particularly troublesome when acetone was used.

*Estimation.* The carbon tetrachloride extract was evaporated to

dryness on the water bath. The neck of the flask was closed with a ground-in tap funnel (the joints were ground so that the flasks would fit either the Soxhlet extractors or the ground-in tap funnel), Fig. 5. The flask was evacuated and the tap closed. For 0.1 gm. sulphur 10 c.c. *N*/1 caustic soda was added through the tap without admitting any air and the flask was replaced on the water bath and left there until all the sulphur had dissolved. In this way any loss of volatile sulphur compounds was avoided. The oxidation was carried out by means of hydrogen peroxide, or, more readily, by potassium bromate and hydrochloric acid, as in the method of Treadwell and Mayr<sup>(13)</sup>. The sulphate formed was estimated by one of the recognised methods.

*Absence of sulphuretted hydrogen and sulphides.*

In all the experiments described tests were made for sulphuretted hydrogen and sulphides in three ways: (a) by hanging a lead acetate paper just above the soil-sulphur mixture, (b) by moistening such a paper with the extract, and (c) by placing a little soil-sulphur mixture on the paper and carefully dropping enough water on the soil to cause it to adhere to the paper. The paper invariably remained white; and numerous other experiments not referred to also gave the same negative results. The temperature varied from 0° to 30° C.; the soil varied from the typical Rothamsted field soil through a rich allotment soil from Rothamsted to the peaty sandy Ormskirk soil, and aëration varied down to complete water-logging for more than a week on end. It seems unlikely, therefore, that sulphuretted hydrogen or other sulphides are ever formed under field conditions in sufficient quantity to have any appreciable toxic action on the Wart Disease fungus, especially in view of their low degrees of toxicity.

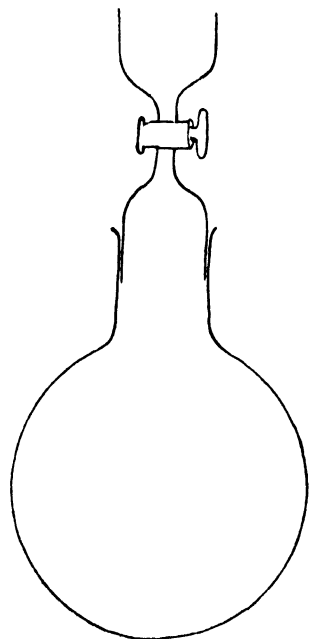


Fig. 5. Oxidation flask for method of Treadwell and Mayr.

*Absence of persulphates, etc.*

Numerous tests made on soil-sulphur mixtures kept under even more widely varying conditions gave no clear indications of the formation of

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any substance able to liberate iodine from slightly acidified potassium iodide; whenever the blue starch-iodide colour was given in a paper test over sulphur-treated soil or in air aspirated through such soil a similar colour was also given when untreated soil was substituted for the sulphur-treated soil. In no single experiment carried out at temperatures likely to be met with in the soil (say up to 30° C.) was there any difference in the behaviour of untreated and sulphur-treated soils as judged by the intensity of the blue colour developed. In spite of the high toxicity of persulphuric acid it seems unlikely that enough could have been formed under these conditions to explain the toxicity of the sulphur. In experiments carried out entirely in solution on soil extracts the results were more definitely negative. A blue colour was never obtained on acidifying and adding potassium iodide and starch solution to a colourless soil extract.

Compounds of classes (i) and (iii) therefore, are not further dealt with in this communication.

### *Evidence for the formation of compounds intermediate in state of oxidation between sulphur and sulphuric acid.*

The following compounds, among others, belong to this class: thio-sulphuric acid,  $\text{H}_2\text{S}_2\text{O}_3$ ; pentathionic acid,  $\text{H}_2\text{S}_5\text{O}_6$ ; tetrathionic acid,  $\text{H}_2\text{S}_4\text{O}_6$ ; trithionic acid,  $\text{H}_2\text{S}_3\text{O}_6$ ; dithionic acid,  $\text{H}_2\text{S}_2\text{O}_6$ ; sulphurous acid,  $\text{H}_2\text{SO}_3$ ; and the salts of these acids.

The experimental procedure in testing for the accumulation of these substances in soil-sulphur mixtures was to treat the soil extract with excess of barium chloride, spin down the barium sulphate precipitate on the centrifuge, then treat the solution with potassium chlorate and hydrochloric acid, when a further precipitate would indicate the presence of one or more of these compounds. (It has already been stated that tests for sulphides were uniformly negative.) A number of preliminary experiments of this kind indicated that in the Rothamsted soil to which sulphur had been added, appreciable quantities of the sulphur were oxidised in the course of a few days to compounds which, on oxidation with chlorine or other strong oxidising agents, gave sulphate. On a number of occasions tests were made for thiosulphate and sulphite by precipitating the sulphate with excess of barium chloride, removing the precipitate and titrating with iodine. In all experiments except one 2 drops of decinormal iodine solution were sufficient to give the "starch-iodide" blue; in the one experiment, as will be seen later (p. 82), 0.2 c.c. was necessary. This evidence, though perhaps suggestive,

is insufficient to establish the presence of thiosulphate or sulphite. The fact that the addition and slight combination of iodine was never followed by a further precipitate of barium sulphate proved that if either of these two compounds was present it was thiosulphate, which, as is well known, gives tetrathionate with iodine, whereas sulphite forms sulphate. Whenever an appreciable further precipitate was formed after treatment with a strong oxidising agent, another portion of the solution always gave a white, or sometimes even a yellow, precipitate of sulphur, on the addition of 20 per cent. caustic soda. The solution also gave the other tests characteristic of polythionates. Hence the solutions definitely contained pentathionate and possibly other polythionates, though the lower polythionates cannot be detected by any known qualitative test in the presence of pentathionate. The characterisation of the polythionates in the presence of each other can be done only by quantitative means.

The few results of some work along quantitative lines did not justify the drawing of definite conclusions.

A few of the experiments proving the presence of polythionates in sulphur-treated soil are given in more detail below. In most of these the amount of sulphur added was chosen to correspond with a dressing of 1 ton per acre in the field, *i.e.* 0.1 gm. sulphur was added to 100 gm. of the damp soil.

#### EXPERIMENTAL RESULTS.

##### I. *Experiment begun, 28. viii. 26.*

Rothamsted soil, 5000 gm., mixed with 5 gm. sulphur (as used at Ormskirk in 1925). Temperature, 30° C.

*Untreated soil.* An extract of 100 gm. of soil was made by means of apparatus 1. The filtrate was perfectly clear and neutral in reaction. It gave only a barely perceptible turbidity with barium chloride and with benzidine; hence it was practically free from sulphate. After oxidation with hydrogen peroxide or potassium chlorate and hydrochloric acid and subsequent addition of barium chloride, or benzidine, the solution became only very faintly turbid; hence it was free from all but minute traces of all sulphur compounds.

*Soil and sulphur mixture.* The same results as above were obtained with the aqueous extract made from the sulphur-treated soil immediately after the sulphur was added; hence there was no appreciable immediate reaction between the soil and sulphur.

7. ix. 26 (10 days after beginning of experiment).

Tube No. 37 percolated by method 2 and sulphate determined by Raschig's benzidine method.

1st 80 c.c. of percolate  $\equiv$  5.49 c.c. N/10 NaOH

Next 80 c.c. of percolate  $\equiv$  0.00 " "

5.49 " "

$\equiv$  0.018 gm. sulphur as sulphate,

*i.e.* 18 per cent. sulphur oxidised to sulphate.

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### 8. ix. 26. *Test for forms of sulphur other than sulphur and sulphate.*

Two tubes of soil were percolated by method 2, till the percolate no longer gave a precipitate with barium chloride. Each percolate was treated with excess of barium chloride and the precipitated barium sulphate centrifuged off, washed and weighed.

Weights of barium sulphate: (1) 0.0988 gm., (2) 0.1069 gm. The centrifugate, which was perfectly clear, was boiled with hydrogen peroxide. There was a further small precipitate of barium sulphate, indicating that the solution contained some oxidisable sulphur compound soluble in water.

Weights of barium sulphate: (1) 0.0458 gm., (2) 0.0467 gm. Hence in 11 days at 30° C. about

14 per cent. of the sulphur was in the form of sulphate,

6 per cent. of the sulphur was present as some compound oxidisable by hydrogen peroxide to sulphate,

and the remaining

80 per cent. presumably unchanged.

### II. *Experiment begun, 21. i. 27.*

Rothamsted soil, 0.2 per cent. sulphur. Temperature, 30° C.

#### 2. ii. 27 (12 days after beginning of the experiment).

100 gm. of soil percolated by method 2 and oxidisable sulphur estimated by the method of Treadwell and Mayr.

1st percolate	50 c.c. (12.30–6 p.m. = 5½ hr.)	≡ 7.00 c.c. $N/10$ $Na_2S_2O_3$ .
2nd	40 „ (during night)	≡ 2.55 „ „
3rd	50 „ (10 a.m.–12.30 p.m. = 2½ hr.)	≡ 1.35 „ „
4th	50 „ (12.30–5.30 p.m. = 5 hr.)	≡ 1.02 „ „
5th	50 „ (during night)	≡ 0.22 „ „
		<hr/> 12.15 „ „

12.15 c.c. 0.998  $N/10$   $Na_2S_2O_3$  ≡ 0.0097 gm.  $S_8O_6$  sulphur (or about 0.5 per cent.),  
or 0.015 gm.  $S_8O_6$  sulphur (0.7 per cent.).

#### 8. ii. 27 (20 days after beginning of experiment).

Extracts of two separate tubes made by method 3.

No. 1. 1st 150 c.c. extract treated by Treadwell and Mayr method

≡ 7.3 c.c. 0.998  $N/10$   $Na_2S_2O_3$ ,

or 0.0058 gm.  $S_8O_6$  sulphur, or about 2.9 per cent.

No. 2. 1st 150 c.c. extract decolorised 1 drop but not 2 drops  $N/10$  iodine.

Hence no appreciable quantity of  $S_2O_3$  or of  $SO_3$  was present.

Two tubes extracted in the same way as the above two but more slowly; the extracts being united, mixed and divided into two equal parts.

1st half decolorised 1 drop but not 2 drops of  $N/10$  iodine.

2nd half when oxidised in the Treadwell and Mayr apparatus

≡ 9.1 c.c.  $N/10$   $Na_2S_2O_3$ ,

≡ 0.0073 gm.  $S_8O_6$  sulphur, or 3.6 per cent.

## 9. ii. 27 (21 days after commencement of the experiment).

Two lots of 500 gm. sulphur-treated soil extracted by method 2 a.

Polythionate sulphur:

(1)  $\equiv$  75.1 c.c. N/10 bromine or 0.06 gm.  $S_2O_6^{2-}$  sulphur (6 per cent.);

(2)  $\equiv$  50.0 " " 0.04 " " (4 per cent.).

(1) 50 c.c. solution  $\equiv$  0.20 c.c. N/10 iodine solution;

(2) " "  $\equiv$  0.20 " "

This was the only indication of any appreciable quantity of thio-sulphate or sulphite being formed. This evidence is insufficient on which to base the conclusion that a definite amount of thiosulphate or sulphite was present in the extract.

There is definite evidence therefore that in Rothamsted soil kept at 30° C. pentathionate (accompanied possibly by other polythionates) is formed in quantities up to 6 per cent. of the sulphur added. No evidence was obtained in these experiments of the formation of any appreciable quantities of sulphur compounds intermediate in state of oxidation between sulphur and sulphuric acid in Rothamsted soil kept at 15° and 0° C. respectively.

*Ormskirk soil.* Similar but less extensive experiments were carried out on Ormskirk soil; in none of the experiments was there any evidence of the formation of appreciable quantities of such compounds.

Hence we have definite evidence of only one compound being formed in any appreciable quantity as an intermediate product in the oxidation of sulphur to sulphuric acid, viz. pentathionate; but possibly this was accompanied by other polythionates. None of the polythionates shows any toxicity towards the Wart Disease fungus, however, hence their formation on sulphur-treated soil throws no direct light on the mode of fungicidal action of sulphur; but it does give a hint of a conceivable mode of action. It is well known that polythionates are formed when a thiosulphate is acidified, hence the question arises: was the pentathionate found in the soil solution formed from pre-existing thiosulphuric acid? *i.e.* does the oxidation take place in some such steps as the following:

sulphur  $\rightarrow$  thiosulphuric acid  $\rightarrow$  polythionic acids  $\rightarrow$  sulphuric acid.

Much support may be found for this view in the work on the oxidation of sulphur in soil and other habitats and by micro-organisms in culture fluids reviewed by Joffe<sup>(8)</sup> and Guittonneau and Keiling<sup>(6)</sup>. Probably all of these changes are brought about mainly by micro-organisms, but chemical evidence, as reviewed by Bassett and Durrant<sup>(1)</sup>, also supports the view that some such series of changes as the above takes place.

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The fact that the presence of thiosulphate in appreciable quantities was not definitely established by the foregoing experiments is not evidence against the above view. The amount accumulating in the soil solution will be conditioned both by the rate of its formation from sulphur and by the rate of its change into pentathionate. If, now, the soil be extracted, the sulphur is removed with the soil from the reaction solution and the source of the thiosulphate disappears; but there is no reason for supposing that the change of thiosulphate into pentathionate is interfered with materially; thus there is reason for supposing that the amount of thiosulphate in the solution will decrease immediately the sulphur is separated from it. Hence to test for its formation with any degree of certainty it would be necessary either (a) to remove the thiosulphate as an insoluble compound actually from the soil solution itself, (b) to convert it into a compound not changed to pentathionate, or (c) to arrest all these changes suddenly such as by very low temperature and subsequent extraction with alcohol. An opportunity to carry out such experiments did not present itself; but recently work has been done which has shown that thiosulphuric acid can exist in a free state; a comparison has been made between the degree of toxicity of certain solutions and their content of thiosulphuric acid. This helps to assess the value of the suggestion that sulphur acts as a soil fungicide through the formation of thiosulphuric acid.

### *The existence in a free state and the degree of stability of thiosulphuric acid.*

The existence of thiosulphuric acid in a free state is commonly denied in text books, thus Ephraim(3) states: "the free acid has not been isolated, as it decomposes at once into sulphurous acid and sulphur," but, as already indicated in a former paper ((12), p. 181), even after 12 hours an S/1000 acidified thiosulphate solution remained perfectly clear. Even much stronger solutions remain clear for shorter lengths of time. Though acidified thiosulphate decomposes in other ways giving rise to products other than sulphur and sulphur dioxide(1), these facts do rather suggest that the decomposition of free thiosulphuric acid is not instantaneous but requires a definite period of time for completion and that the final equilibrium solution in each case will contain definite amounts of free thiosulphuric acid. Before this idea could be tested analytical methods have had to be modified for the purpose.

The readiest method of estimating thiosulphate is by titration with standard iodine solution, but this method is not applicable unmodified in the presence of sulphite, since sulphite also is oxidised by iodine.

The sulphite may be precipitated and removed, or more simply it may be locked up by treatment with formaldehyde in a compound in which it is not acted upon by iodine; the thiosulphate may then be titrated with iodine (assuming sulphides to be absent, as in fact they were in all the solutions examined). The necessary conditions for concentrated solutions were worked out by Kurtenacker (9). Certain refinements, however, are necessary before the method is applicable to dilute solutions. The modified method will be described before giving the results obtained by it.

*Estimation of thiosulphate in presence of sulphite by a modification of Kurtenacker's method.*

The essence of Kurtenacker's method is to neutralise the solution to phenol phthalein with ammonia to remove any strong acids, add an excess of formaldehyde, then render acid with acetic acid and titrate immediately after acidification with iodine solution. Under these conditions the sulphite is rendered almost completely inactive towards iodine, the excess formaldehyde has no measurable effect on iodine and any formaldehyde thiosulphuric acid formed is sufficiently unstable to allow of rapid titration of the thiosulphate part of it.

The method exactly as recommended by Kurtenacker was found satisfactory for the relatively concentrated solutions with which he worked, but in more dilute solutions it became inapplicable. The following modifications were found necessary or considered desirable.

*Excess of potassium iodide.* For iodine-thiosulphate titrations in *N*/10 solution the amount of the excess of potassium iodide added does not matter within limits, though even in such concentrated solutions a large excess causes the colour with starch to be brown rather than blue and to fade rather rapidly making the end-point somewhat indefinite. No experimental evidence could be found for the desirability of the addition of any potassium iodide besides that usually contained in the iodine solution. As will be seen later, the error, through omitting to add further potassium iodide, is only a small fraction of a drop of *N*/10 iodine solution. The influence of the concentration of potassium iodide in the solution titrated is well seen by determining the amount of standard iodine solution necessary to give a definite colour in presence of varying amounts of potassium iodide.

Each solution contained 50 ml. water, 1 ml. 0.5 per cent. starch, 1 ml. glacial acetic acid.

ml. 10 % KI added	0	1	5	10
1 drop <i>N</i> /100 iodine	No colour	Pure blue	Not pure blue	Reddish blue
2 drops	Faint pure blue	—	—	—
3 drops	Definite pure blue	—	—	—

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Thus 2 drops of  $N/100$  iodine are sufficient to give a definite though faint coloration in 50 ml. solution and 3 drops an easily recognised blue colour, without the addition of any potassium iodide other than that in the iodine solution. The addition of 1 ml. 10 per cent. potassium iodide to 50 ml. solution (*i.e.* 0.2 per cent. KI) was sufficient to cause 1 drop of  $N/100$  iodine solution to give a definite pure blue colour. The colour became more and more reddish as more potassium iodide was added, becoming brown and finally practically disappearing when large excesses were added.

With  $N/1000$  iodine using the same solution as the above the following results were obtained:

ml. 10 % KI added	0	0.25	0.5	1
1 drop $N/1000$ iodine	Colourless	Colourless	Colourless	Colourless
5 drops     "	"	Light blue	Light blue	Light blue
10 drops   "	"	Blue*	Blue*	Blue*
25 drops   "	Just perceptible colour	—	—	—
30 drops   "	Blue*	—	—	—

Solutions marked (\*) were of approximately the same colour. On the further addition of 5 ml. 10 per cent. KI the blue colour became tinged with red but was hardly any deeper.

Hence the presence of 0.2 per cent. potassium iodide was sufficient to cause 1 drop of  $N/100$  iodine, to give an easily distinguishable blue colour and 5 drops of  $N/1000$  iodine to give a definite light blue colour. This concentration was accepted as the best for the present purpose.

*Excess of formaldehyde.* Experiments were carried out to determine the minimum amounts of formaldehyde necessary to render inactive towards iodine the various amounts of sulphite likely to be dealt with. Thus using 1 ml. 35 per cent. formaldehyde in 50 ml.  $S/200$  sodium sulphite (+ 1 ml. glacial acetic acid, 1 ml. 10 per cent. KI, 1 ml. 0.5 per cent. starch), 1 drop of  $N/100$  iodine gave a definite colour. With quantities of formaldehyde less than 1 ml. more than 1 drop of  $N/100$  iodine was necessary.

In order to determine whether this amount of formaldehyde had any serious effect on the iodine titration under the experimental conditions the following solutions were made up:

**A**

50 ml. water  
1 ml. 35 % formaldehyde  
1 ml. 10 % KI  
1 ml. glacial acetic acid  
1 drop  $N/100$  iodine  
1 ml. 0.5 % starch solution

**B**

50 ml. water  
1 ml. 10 % KI  
1 ml. glacial acetic acid  
1 drop  $N/100$  iodine  
1 ml. 0.5 % starch solution

The colour of solution *A* was visible even without using a beaker of pure water for comparison; hence the difference due to an excess of 1 ml. 35 per cent. formaldehyde was less than 1 drop of *N*/100 iodine.

The colour of solution *A*, however, was fainter than that of solution *B*; hence even this small amount of formaldehyde had some small effect on the iodine, so it was considered undesirable to increase the amount more than necessary. This amount was found satisfactory for *S*/20, *S*/100 and *S*/1000 solutions.

The modified method, therefore, is as follows: to 25 or 50 ml. solution are added (in the following order) enough ammonia to render neutral to phenol phthalein, 1 ml. 35 per cent. formaldehyde, 1 ml. 10 per cent. potassium iodide and 1 ml. glacial acetic acid; immediately after the addition of the acetic acid the solution is titrated with iodine.

The following results demonstrate the degree of accuracy readily attainable:

1. 25 ml. *S*/100  $\text{Na}_2\text{S}_2\text{O}_3$  + 25 ml. water + 1 ml. glacial acetic acid + 1 ml. 10 per cent. potassium iodide + 1 ml. 0.5 per cent. starch  
 $\equiv (12.45), (12.44), 12.49, 12.49 \equiv 12.49$  ml. *N*/100 iodine solution.
2. The above solution + 1 ml. 35 per cent. formaldehyde  
 $\equiv 12.49$  ml. *N*/100 iodine solution.
3. Solution 2 with water replaced by 25 ml. *S*/100  $\text{Na}_2\text{SO}_3$   
 $\equiv 12.49, 12.51 \equiv 12.50$  ml. *N*/100 iodine solution.

By the time the above analytical method had been worked out it was no longer possible to test the toxicities of solutions simultaneously with their analysis. The best that could be done therefore was to analyse solutions made up to be as similar as possible to certain of those the toxicities of which had already been determined (12). In that paper (p. 181) are given the toxicity figures for a double series of solutions of thiosulphuric acid in the presence of excess of sulphuric acid in the one series and of sulphurous acid in the other series.

When a thiosulphate is acidified a variety of compounds are formed which exist more or less in equilibrium with each other. As is well known, if to such a decomposing compound one of its products of decomposition is added, the decomposition tends to be arrested and so the equilibrium mixture tends to contain more of the undecomposed compound. Thus sulphurous acid being known to be a decomposition product of thiosulphuric acid it might be expected that the substitution of sulphurous for sulphuric acid in the solution of thiosulphuric acid

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would result in changes in the equilibrium solution obtained. In particular the relation between any undecomposed thiosulphuric acid and its decomposition products would be upset. That some such modification of the equilibrium mixture was brought about is shown by the fact that

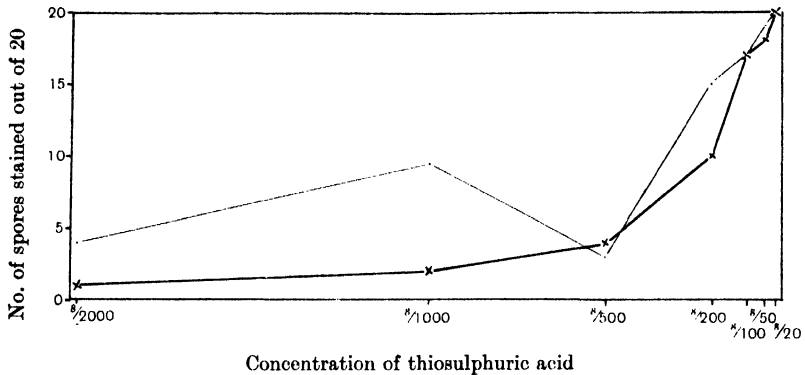


Fig. 6.

Connection between concentration and toxicity of thiosulphuric acid solutions in presence of excess of sulphuric acid .....•....., sulphurous acid —x—x. Spores treated for 24 hours. Results taken from Roach and Glynn (12).

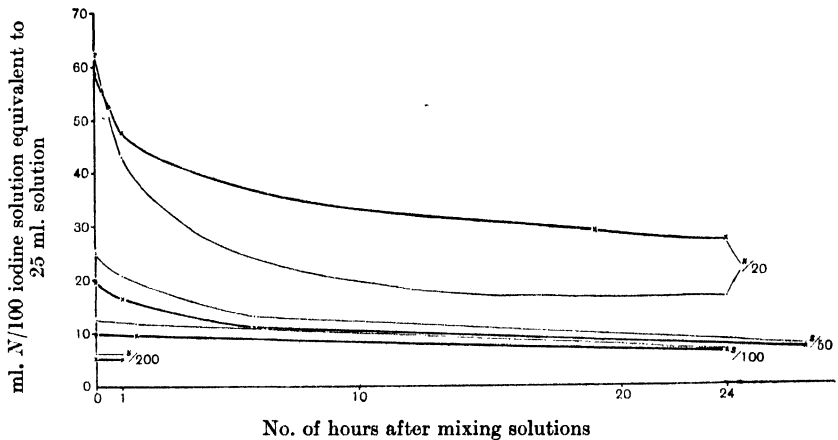


Fig. 7.

Concentration of thiosulphuric acid after varying lengths of time in presence of excess of sulphuric acid .....•....., sulphurous acid —x—x.

the separation of sulphur was different in the two series of solutions, hence a comparison of the toxicities of such solutions with their thiosulphuric acid contents might be expected to throw light on the question of the degree of connection between them. As may be seen from Fig. 6 toxicities of corresponding solutions of the two series are the same

within the admittedly large experimental error. Similar solutions were made up and their thiosulphuric acid contents determined after standing for varying lengths of time. The analytical results are shown in Table I and Fig. 7. The figures given are the numbers of ml. *N*/100 iodine solution equivalent to 25 ml. of the solution tested. As a check an aliquot portion of each solution was titrated before the acid was added and in addition some of the solutions were neutralised and titrated immediately after the acid was mixed. It will be seen from Table I that there is no justification for the general belief that thiosulphuric acid is completely decomposed as soon as liberated; for at the end of the few minutes necessary to mix the acid and thiosulphate solutions, pipette off the aliquot portion and neutralise it, less than 1 per cent. of the thiosulphate had disappeared.

Table I.  $\text{H}_2\text{S}_2\text{O}_3$ .

	<i>S</i> /20		<i>S</i> /50		<i>S</i> /100		<i>S</i> /200	
	Excess $\text{H}_2\text{SO}_4$	Excess $\text{H}_2\text{SO}_3$	Excess $\text{H}_2\text{SO}_4$	Excess $\text{H}_2\text{SO}_3$	Excess $\text{H}_2\text{SO}_4$	Excess $\text{H}_2\text{SO}_3$	Excess $\text{H}_2\text{SO}_4$	Excess $\text{H}_2\text{SO}_3$
Before mixing	62.4	58.6	25.25	19.2	12.65	9.96	6.265	5.2
Immediately after mixing	61.94	58.4	25.05	—	12.6	—	—	—
$\frac{1}{4}$ hr. after mixing	56.0	55.65	—	—	—	—	—	—
$\frac{1}{2}$ hr. "	51.1	52.2	—	—	—	—	—	—
1 hr. "	43.0	47.4	20.01	16.5	—	—	6.21	5.15
$1\frac{1}{2}$ hr. "	—	—	—	—	11.9	9.95	—	—
19 hr. "	—	29.0	13.0	11.0	—	—	—	—
24 hr. "	16.5	27.25	—	—	6.9	6.3	—	—
27 hr. "	—	—	7.65	7.0	—	—	—	—
44 hr. "	10.0	—	—	—	—	—	—	—
7 days after mixing	—	—	3.35	2.35	2.7	2.35	2.83	2.8

Next, the increase of stability of the thiosulphuric acid with dilution will be noticed; thus the titration figure for the *S*/20 thiosulphuric acid with excess of sulphuric acid in 24 hours decreased from 62.4 to 16.5 (*i.e.* roughly 26 per cent. left undecomposed), whereas the corresponding *S*/100 solution only decreased from 12.65 to 6.9, *i.e.* 55 per cent. left undecomposed. Results at the end of 7 days lead to the same conclusion. Whereas the figure for the *S*/50 solution decreases from 25.25 to 3.35 (*i.e.* 13 per cent. undecomposed), the *S*/200 solution only decreases from 6.265 to 2.83 (*i.e.* 45 per cent. still undecomposed).

Bearing in mind the fact that the solution in which the excess acid was in the form of sulphurous acid was poorer in thiosulphuric acid at the start than those in which the excess acid was sulphuric acid, the figures show that the substitution of an excess of sulphurous for an excess of sulphuric acid has resulted in a slight increase of stability of the thiosulphuric acid; but taking into account the inaccuracy of the

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toxicity figures, this increase in stability is insufficient to affect the toxicity figures appreciably, except perhaps for the *S*/20 solutions; as both of these are completely toxic under the conditions of the test, any such difference cannot show itself.

The toxicity, therefore, varies with the thiosulphuric acid content as far as can be judged from the result of this test, which, as it turns out, is admittedly not a very searching one. Even though the replacement of the excess of sulphuric acid by the excess of sulphurous acid did not result in any great change in thiosulphuric acid content, the difference in sulphur deposition (12), p. 181) shows that the equilibrium of the solutions was definitely upset in other ways. The toxicity figures following the thiosulphuric ones and not the sulphur deposition make the connection between the toxicity figures and thiosulphuric acid content the more likely to be a causal one. The decomposition of the thiosulphuric acid becomes very slow when it reaches a concentration of about *S*/100 or lower. This solution is almost completely toxic in 24 hours. This fact may explain why *S*/20 thiosulphuric acid remained completely toxic when the spores were exposed for 24 hours even when tested 7 days after the solution was made up, and why an *S*/200 solution had approximately the same toxicity whether tested immediately after the solution was made up, 1 day or 7 days afterwards, as stated on pp. 179 and 180 of the previous paper (12).

We can test still further the idea that sulphur owes its fungicidal action in soil to the formation of thiosulphuric acid; we can see to what extent the minimum toxic concentration of thiosulphuric acid found in the laboratory fits in with the minimum effective dressing of sulphur in the field.

An exposure of Wart Disease spores to an *S*/200 solution of thiosulphuric acid for 10 days results in about 19/20 being killed (12), p. 178). It has been calculated that a dressing of about 11 cwt. sulphur per acre can free the soil from disease in the ensuing season (10). We can, therefore, say that these two treatments are roughly equivalent in their effects on the disease-producing organism. 1000 c.c. *S*/200 solution of thiosulphuric acid contains

$$\frac{32}{200} = 0.16 \text{ gm. sulphur.}$$

11 cwt. per acre is roughly equivalent to 0.055 per cent. on the top 9-inch soil; assuming a 20 per cent. soil mixture content, 1000 c.c. soil solution will contain  $0.055 \times \frac{100}{20}$

$$= 2.75 \text{ gm. sulphur.}$$

For 1000 c.c. of solution to be completely toxic we have seen that it must contain 0.16 gm. sulphur as thiosulphuric acid over a period of 10 days, *i.e.*  $\frac{0.16 \times 100}{2.75}$ , or roughly 6 per cent. of the sulphur added.

It is not likely that the fungicidal action of sulphur would ever be over in so short a time as 10 days; if not, the above figure of 6 per cent. must be reduced, but against this must be placed the fact that the thiosulphuric acid has to reach each spore by diffusion in at least the minimum toxic concentration, seeing that it is continually decomposing it must start off at the source, the sulphur particle, at a somewhat high concentration.

After preparing this communication for publication the writer has read the criticisms made by Williams and Young (14) of deductions drawn in an earlier paper (12). They state: "Recent work by Roach and Glynne was interpreted by them as pointing to thiosulfuric acid as a toxic factor. However, since the thiosulfuric acid is a very unstable acid and since the condition of their experiments were such as to insure the presence of polythionic acids, the toxicity which they measured was undoubtedly that of the polythionic acids. Furthermore, the tables given by them show a notable toxicity of pentathionic acid itself when compared with other acids tested."

Facts reported on pp. 85-92 of this paper are a sufficient answer to that part of their criticism which is based on the supposed complete instability of thiosulphuric acid.

The sample of barium pentathionate used ((12), p. 188) was exceptionally pure (over 90 per cent.), and in making up the pentathionic acid solutions allowance was made for the 10 per cent. impurity which was probably water. The dilute solutions, when made up, were free from all but traces of sulphite and thiosulphate<sup>1</sup> and remained so the whole period of the tests (up to 10 days). The toxicities of these solutions therefore probably were a fair measure of that of pentathionic acid. The present writer cannot agree with Williams and Young's statement: "the tables...show a notable toxicity of pentathionic acid itself when compared with other acids tested" and sees no reasons for departing from the original conclusion ((12), p. 175): "The three polythionic acids were of the same order of toxicity as sulphuric acid" (see Fig. 8 in which the relevant data are reproduced). Even assuming, what may actually be true, that pentathionic acid has a slightly higher toxicity than sulphuric acid, it is definitely less toxic than thiosulphuric acid, the difference being outside the large limit of experimental error of the tests. Whatever the cause of the toxicity of acidified thiosulphate solutions it can hardly be through the formation of pentathionic acid which is of lower toxicity when compared on a sulphur basis.

That thiosulphuric is the factor chiefly, if not entirely, responsible for the toxicities of the sulphur compounds considered (over and above that due to the hydrogen ion) seems to offer the simplest explanation and the one most free from contradictory

<sup>1</sup> Williams and Young give the results of no such tests to establish the purity of the compounds used in their work. The writer found it necessary to prepare many samples and purify repeatedly before one even relatively free from thiosulphate was obtained.

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facts and unproven assumptions; hence it is retained unless and until a better one presents itself. The difficulties and uncertainties connected with the chemistry of sulphur compounds make anything approaching a final conclusion at this stage unjustifiable.

As already stated, the above tentative conclusion referred to Wart Disease only and does not necessarily relate to any other fungus.

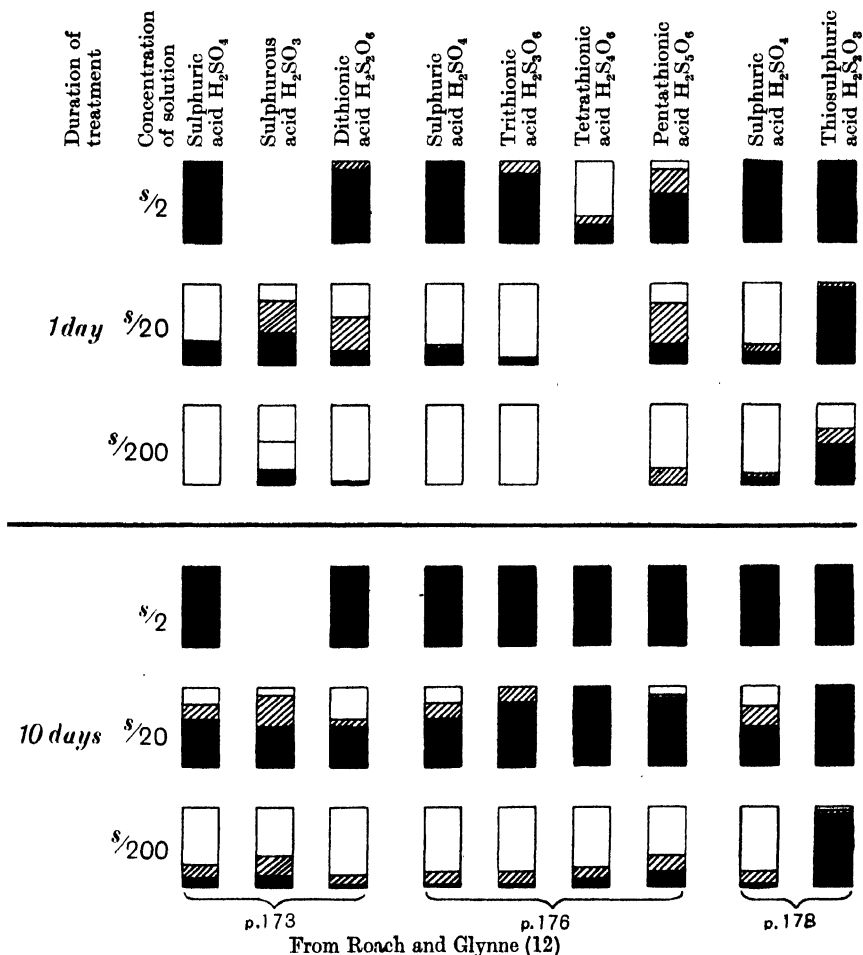


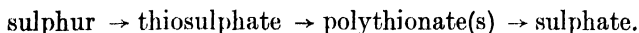
Fig. 8. Toxicities of various sulphur acids towards *Synchytrium endobioticum*.

Key to diagram:

- Sporangia stained faintly and therefore alive.
- Sporangia stained to intermediate degree and probably dead.
- Sporangia stained deeply and therefore dead.

## DISCUSSION.

It was suggested on p. 83 that the oxidation of sulphur to sulphate in soil-sulphur mixtures may take place in the following stages:



There seems little doubt that sulphate is the end-product under a variety of conditions. Pentathionate has been proved in the present investigation to be formed, and to accumulate, in sulphur-treated Rothamsted soil kept at 20° C., but, even if formed under the other conditions tried, it did not accumulate appreciably. These observations have been confirmed by Hobson (see Appendix). He has also shown that thiosulphate when added to soil is changed first into pentathionate and then into sulphate. Of the change of sulphur into thiosulphate, the present investigation offers no more than a mere suggestion (p. 82), but Guittonneau and Keiling(4, 5, 6, 7) proved that thiosulphate accumulated in soil-sulphur mixtures under the conditions of their experiments. Moreover, they showed that the addition of peptone greatly increased the accumulation of this substance, thus proving that the degree of accumulation is affected by the chemical composition of the soil solution.

These facts afford sufficient indications of the sensitiveness of the above series of chemical changes to physical and chemical conditions. They suggest the possibility that under the conditions of 1925 and previous years, when sulphur treatment was effective in controlling Wart Disease in the succeeding crop of potatoes, the oxidation of the sulphur may have taken place in such a way that there was a temporary accumulation of thiosulphuric acid sufficient to make the soil solution toxic to the fungus, whereas under the colder and in other ways different conditions of 1926, when the disease was apparently unaffected, there was little or no accumulation of this substance.

This explanation is the only one put forward at present at all in harmony with the facts; but it cannot be regarded as established unless and until a definite connection has been proved between the amount of thiosulphuric acid accumulating in the soil and the degree of effectiveness of the treatment.

## SUMMARY.

- (1) Thiosulphuric acid has been shown to exist in a free state.
- (2) It is relatively stable in dilute solution; an  $M/200$  solution is only half decomposed at the end of 1 day and an  $M/400$  solution at the end of 10 days only.

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(3) This degree of stability is sufficient to account for the fungicidal action of acidified thiosulphate solutions in terms of the liberated thiosulphuric acid.

(4) It can be calculated that it is only necessary to assume 6 per cent. of the minimum quantity of sulphur found effective against Wart Disease in the field to be in the form of thiosulphuric acid over a period of 10 days in order to account for its toxicity.

(5) Experiments of a preliminary nature carried out on sulphur-treated soil proved the formation of pentathionate in Rothamsted soil kept at 30° C., but not in Ormskirk soil kept at the same temperature, nor in either soil at the lower temperatures of 0° and 15° C.

(6) No definite evidence of the accumulation of appreciable quantities of thiosulphuric acid in the soil was obtained, but reasons are given why this negative evidence is by no means final.

(7) Chemical considerations and the work of others suggest that the pentathionate actually identified in the soil solution arose from thiosulphuric acid formed in an early stage of the oxidation of the sulphur.

(8) The explanation of the fungicidal action of sulphur towards Wart Disease in soil in terms of the formation of thiosulphuric acid is alone in harmony with the ascertained facts.

### APPENDIX.

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After Mr Roach's departure from this laboratory, I continued for a short time the experiments started by him on the decomposition of sulphur in the soil and am taking this opportunity, offered to me by him, of presenting certain results which while of interest do not merit separate publication.

Incubations of sulphur and soil gave results which agree in general with Roach's findings. Sulphate was found to be the main product of the reaction and the only intermediate compounds whose presence could be established with any certainty were the polythionates. In one experiment carried out at 30° C. the amount of polythionates lay between 2 and 7 per cent. of the sulphur added (0.1 gm. per 100 gm. soil) for a period of 30 days. During this time the amount of soluble sulphur rose from 44 to 58 per cent. of the added sulphur. At temperatures of

0° C. and 15° C. polythionates were present only in traces, less than 1 per cent. of the sulphur added.

On one occasion a water extract of sulphur-treated soil gave a blue colour with acidified potassium iodide solution containing starch, which suggested the presence of persulphate. The extract, however, gave no blue colour with an alcoholic benzidine solution, a test which is sensitive to one part of persulphate per million. An extract of untreated soil with dilute sulphuric acid released iodine in the same way, probably through dissolving a metallic oxidising constituent, as aqueous extracts gave negative results. The sulphur-treated soil was alkaline (*pH* 8.3), but it is reasonable to suppose that local acidity in the region of the sulphur particles brought into solution the oxidising agent. There can be no doubt that persulphate was absent.

In order to test the stability of thiosulphate in soil, sodium thiosulphate was added to Rothamsted soil, in amount equivalent to 0.1 gm. sulphur per 100 gm. soil. After 5 days' percolation was carried out and the percolate analysed 5 per cent. of the sulphur was found in the form of sulphate, 84 per cent. as polythionates and only 11 per cent. as thiosulphate. Part of the last may have been present as sulphite which was not estimated separately. The disappearance of the thiosulphate was not due to the instability of thiosulphuric acid in acid solution, as the soil was slightly alkaline in reaction. It seems probable that such a powerful reducing agent cannot exist in the presence of material as labile as soil without undergoing oxidation.

In view of the rapid disappearance of added thiosulphate it is not surprising that thiosulphate could not be detected in sulphur-treated soil. As polythionates were the main transformation product of the added thiosulphate, it seems not unlikely that the small amounts of polythionates found in sulphur-treated soil originated from thiosulphate.

#### REFERENCES.

- (1) BASSETT, H. and DURRANT, R. G. "The inter-relationships of the sulphur acids." *J. Chem. Soc.* (1927), 1401-1468.
- (2) CROWTHER, E. M., GLYNNE, MARY D. and ROACH, W. A. "Sulphur treatment of soil and the control of wart disease of potatoes in pot experiments." *Ann. App. Biol.* (1927), **14**, 422-427.
- (3) EPHRAIM. *Inorganic Chemistry* (1926), Eng. trans. P. C. L. Thorne, Gurney and Jackson.
- (4) GUITONNEAU, G. "Sur la formation d'hyposulfites aux dépens du soufre par les micro-organismes du sol." *C.R. Acad. Sci.* (Paris), (1925), **180**, 1142-1144.
- (5) — "Sur l'oxydation microbienne du soufre." *C.R. Acad. Sci.* (1926), **182**, 661-663.

## 96 *Sulphur as a Fungicide against Potato Wart Disease*

- (6) GUITTONNEAU, G. and KEILING, J. "Sur la solubilisation du soufre élémentaire et la formation des hyposulfites dans une terre riche en azote organique." *C.R. Acad. Sci.* (1927), **184**, 898-901.
- (7) GUITTONNEAU, G. "L'oxydation microbienne du soufre dans ses rapports avec l'évolution de la matière azotée dans le sol." *Proceedings and Papers of the First International Congress of Soil Science convened in Washington, D.C. June 13-22, 1927 for the International Society of Soil Science by the American Organising Committee, Washington, D.C. 1928.*
- (8) JOFFE, J. S. "Biochemical oxidation of sulphur and its significance to agriculture." *New Jersey Agric. Exp. Sta.* (Dec. 1922), *Bull.* 374.
- (9) KURTENACKER, A. "Über Anwendungen der Alderhyd-Bisulfitreaktion in der Massanalyse." *Z. anal. Chem.* (1924), **64**, 56-61.
- (10) ROACH, W. A., GLYNNE, MARY D., BRIERLEY, WM. B. and CROWTHER, E. M. "Experiments on the control of Wart Disease of Potatoes by soil-treatment with particular reference to the use of sulphur." *Ann. App. Biol.* (1925), **12**, 152-190.
- (11) ROACH, W. A. and BRIERLEY, WM. B. "Further experiments on the use of sulphur in relation to Wart Disease of Potatoes." *Ann. App. Biol.* (1926), **13**, 301-307.
- (12) ROACH, W. A. and GLYNNE, MARY D. "The toxicity of certain sulphur compounds to *Synchytrium endobioticum*, the fungus causing Wart Disease of Potatoes." *Ann. App. Biol.* (1928), **15**, 168-190.
- (13) TREADWELL, F. P. and MAYR, C. "Eine neue Methode zur jodometrischen Bestimmung der Rhodan- und Schwefelwasserstoffsäure." *Z. f. anorg. Chem.* (1915), **92**, 127-134.
- (14) WILLIAMS, ROBERT C. and YOUNG, H. C. "The toxic property of sulfur." *Ind. Eng. Chem.* (1929), **21**, 359-362.

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## EXPERIMENTS WITH A MOSAIC DISEASE OF TOMATO

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(With Plate IX.)

*Description of Virus.* In the experiments described in the following pages a virus was used which produces in the tomato a mosaic disease somewhat different in appearance from the usual tomato or tobacco mosaic. It was given to the writer in 1925 by Dr W. F. Bewley, who had found it on tomato two or three years previously, and had given it the name of Aucuba mosaic of tomato from its resemblance (not, it must be admitted, very close) to Aucuba mosaic of the potato. In its general characters as well as in the nature of the disease it produces, it corresponds very closely with the virus of tomato or tobacco mosaic, but it differs from the latter in the much greater intensity and brilliance of the leaf-symptoms. Since its first isolation it has been maintained regularly on tomato without change in these symptoms, and the difference, while possibly only one of degree, is so striking that it is difficult to avoid the conclusion that there is also a difference in the two viruses.

It seems probable that this mosaic is the same as the "Yellow Tobacco Mosaic" (Tobacco Virus 6) first described by Johnson in 1927(8) and again referred to by Hoggan(7). The characters of the virus, so far as they are known, are the same in both, and the signs produced by yellow mosaic on tomato and tobacco, as shown in the figures in Johnson's paper, resemble very closely those produced by the virus here described. Without further work, however, and until a more satisfactory system of virus classification has been established, it is impossible to be certain that the two are identical.

While the signs on the plant vary somewhat with the variety of tomato and still more with external conditions of temperature and light, and the rate of growth, the following description of it as it occurs on Kondine Red tomatoes is fairly typical of the disease in general. When the plants are very young at the time of inoculation, *i.e.* with only about three leaves large enough to be readily inoculated, and the

conditions are favourable for rapid growth, the first signs appear in the young developing leaves of the crown about the 5th day. These show a downward curling of the whole leaf, with slight turning down of the margins, and the surface of the leaf is rough, wrinkled or corrugated. The colour is still green with no sign of chlorosis or mottling, but the evidence of abnormal growth is quite definite. By the 7th or 8th day points of chlorosis appear on these curled leaves, sometimes at the base of the leaflet, more usually at the tips and margins, and these rapidly increase in number, are distributed over the whole surface of the leaf, and tend to coalesce. By the 12th or 13th day, when usually six more leaves have unfolded, the signs of disease differ in the different leaves. The original three may still show no chlorosis, or only a slight yellowing of the veins, and the youngest leaf may also be quite green. But the fourth and fifth and sometimes the sixth, *i.e.* the leaves developing after inoculation, are extensively affected. In extreme cases almost the whole surface is pale yellow to white with here and there small islets of intense dark green, which stand up as small blisters. In less extreme cases the green areas are larger, but as a rule the area of white or pale yellow is greater than the green area. The surface is uneven and there may be turning down of tip and margins. These three leaves show the most extreme form of the disease which will be seen in the whole plant. The younger leaves at this time show only scattered patches of white or yellow, frequently angular or triangular at vein intersections, and the youngest leaf may be entirely green. As growth proceeds each leaf in turn may come out with only slight colour changes, but later on each develops more or less extensive signs, though rarely in the later leaves is the chlorosis so extensive as in the fourth to the sixth leaves. When the plant reaches a height of 18 to 24 in., by which time the first flowers are forming, a typical leaf will show on each leaflet areas of four different shades. Most of the surface is green, partly of normal tint and partly of a deeper and richer shade. Scattered over the leaf are patches of white and patches of yellow, usually sharply delineated but sometimes shading into neighbouring areas, irregular in shape and size, often angular, and occurring in all parts of the leaf (Plate IX, fig. 1).

The plant is not killed, but goes on to the production of fruit, which may or may not be mottled. But its growth is checked: compared with normal plants of the same age, it is stunted and of spindling habit. There is no necrosis. Sometimes the extensive chlorotic areas, especially in the fourth to sixth leaves, dry out, and turn a dull brown, but necrosis is not a character of the disease, and in many cases even this secondary

bronzing does not occur. There is little tendency to extreme malformation, though quite definite fern-leaf has been noted occasionally on plants growing rather slowly, *e.g.* in the autumn.

In atypical cases, or when incubation is unusually prolonged or the disease is less acute, the leaves may show at first a yellowing of the veins, which thereby become more conspicuous, and appear as a yellow network on a green background (Plate IX, fig. 2). As a rule these leaves later develop intervenal signs. If the plant is already well grown, *i.e.* with flowers already out, at the time of inoculation, the signs appear on the younger leaves, and not on the older parts, incubation is prolonged to 14 days or more, and there is not the extensive whitening seen on the plant which has been infected young. The signs are, however, perfectly clear and definite, and of the same character as those in the later-developing leaves of the plant infected young.

From this description it will be seen that the course of the disease resembles very closely that of the usual mosaic on tomato or tobacco. The character of the symptoms also on the whole resembles the more common disease, and, as is shown later, this virus is like the ordinary mosaic in its filterability, resistance to heat, to alcohol, to dilution and to ageing, and in its ready transmissibility by inoculation of juice or tissue as well as by insects. But the actual picture presented by the *Aucuba* or yellow type is much more striking, more spectacular, than any which the writer has seen with the usual mosaic. The white areas are more intensely white, the green areas more vividly contrasted, and there is usually a sharper delimitation between the two. When the two types are seen side by side, the difference is very conspicuous. Essentially, however, the two diseases are the same in kind throughout, and the *Aucuba* type has been used in these experiments as a typical mosaic disease, because it is very characteristic, easily recognisable, and not liable to be confused with the mottlings due to physiological or environmental conditions or with possible contamination by other types of mosaic.

*Methods of Inoculation.* Inoculation was made at first by simple pricking with a needle through juice dropped on the leaf, a total of 80–100 punctures being made on at least three leaves per plant. In later work this method has been modified by supporting the leaf on the wooden slip used to mark the pot, dropping on it the inoculum, and then scratching through the drop with the point of a needle in a number of places, usually about 30 per leaf and always inoculating at least three leaves. This method avoids all contact of the hands with the leaf or

inoculum, and is effective. The weakness of all such methods is that one has very little idea of the amount actually inoculated and not much assurance that any two plants have received the same dose, whatever its size. The variation, however, can hardly be greater than 100 per cent., and an accuracy of this order is as great as is necessary in most work.

In a number of cases infection was carried out in a different manner. A petiole was cut across, and the cut end dipped into a small phial containing the juice to be inoculated. The plant absorbs the contents of the phial through the cut surface, and in this way a definite dose can be introduced. But the method is too laborious for use with large numbers of plants. It is also rather uncertain, since one plant may take up in one hour as much as 1 c.c., while another takes up only 0.2 c.c., and sometimes only quite small quantities may be taken up in 18 hours. Further, it would seem to be less efficient. In one series of four plants absorption of 0.5 c.c. of filtered juice by each, and in another series of six plants absorption of 0.7 c.c. by each failed to produce the disease in any, while in a third series only 50 per cent. of the plants took the disease as against 83 and 100 per cent. in two other series done at the same time with different methods.

*Method of Filtration.* The method adopted to obtain filtered juice is as follows. Leaves and succulent parts of the stem of infected plants are weighed, minced with scissors, and ground in a mortar without sand, distilled water added gradually with renewed grinding in the quantity of 3 c.c. of water to 1 gm. of tissue, and the mass squeezed by hand through muslin. The resulting turbid green liquid is then passed, under pressure, through a cylinder tightly packed with alternate layers of sand and paper-pulp<sup>1</sup>, which gives a perfectly clear brown fluid. (At one time instead of the cylinder filter-paper was used, the liquid being passed through the same paper more than once, but this was a slow and uncertain process.) The clear fluid is then passed through first a "L 1," and then at once through a "L 3" Pasteur-Chamberland filter; and the filtered fluid immediately distributed in small volumes (5 or 10 c.c.) into test-tubes or flasks, and remains bacteriologically sterile. The whole process from cutting the plant to final distribution of 150-250 c.c. of fluid requires about 4 hours: all apparatus having been previously sterilised. Unless otherwise stated, "filtered juice" in this paper means juice prepared in this way. From 100 gm. of tissue to which 300 c.c. of water are added, 330-340 c.c. of crude liquid are obtained after

<sup>1</sup> This should be made from macerated ashless filter-paper; the usual commercial compressed pulp alters the reaction of the juice.

squeezing through muslin. Some liquid, of course, remains in the mass of tissue, but one may reckon that the juice is diluted 1 in 8 to 1 in 10 before passing through the candles.

*Effect of Dilution.* In Table I is shown an experiment to determine to what extent filtered juice can be diluted and still remain infective. The dilution was made with distilled water, a fresh pipette being used for each step. It will be seen that 1 in 1000 is still fully infective; 1 in 10,000 infective but less markedly; with 1 in 100,000 or weaker strengths infection did not occur. This is the usual result with juice obtained from young plants grown under standard conditions and with the signs of disease well-marked, about 4 weeks after infection; but some variation occurs under other conditions, *e.g.* when the plants are less succulent or older. Also, the 1 in 100 dilution may not always produce 100 per cent.

Table I.

*Filtered juice diluted in distilled water.*

Dilution	No. of plants	No. positive	% positive
1 : 10 <sup>2</sup>	8	8	100
1 : 10 <sup>3</sup>	8	8	100
1 : 10 <sup>4</sup>	8	3	37.5
1 : 10 <sup>5</sup>	8	0	0
1 : 10 <sup>6</sup>	8	0	0

infection, not even when a dilution of 1 in 10,000 is still partially infective; but with 1 in 100 dilution infection of over 80 per cent. was always obtained during the season of the year favourable to growth. These results correspond well with those obtained with the usual tobacco mosaic virus. With it, and using unfiltered juice, infection may still be got with dilutions of 1 : 100,000 or even 1 : 10<sup>6</sup> (Allard<sup>(1)</sup>), but filtration always reduces notably the extent to which dilution is practicable.

*Resistance to Heat and Ageing.* The filtered juice withstands heating for 10 minutes at 80° C. but is no longer infective after exposure for the same time at 90° C. (Table II). These limits are no doubt subject to a certain amount of variation, according to the composition of the particular juice or other factors, and also according to the concentration of virus in the sample under test. No definite thermal death-point is possible, since there is involved a time-factor which varies with the concentration of the material undergoing destruction. But with standardised plants and uniform methods inactivation occurs regularly between 80° C. and 90° C. in 10 minutes. No attempt has been made to obtain more precise determination.

Table II.

2.5 c.c. samples of filtered juice in thin-walled tubes, with thermometer attached, introduced into water-baths at the temperatures named, and withdrawn to cold water after 10 minutes. Great care was taken to ensure that the whole sample was deeply immersed.

Temp. (° C.)	No. of plants	No. positive	% positive
50	7	7	100
60	7	7	100
70	8	8	100
80	8	8	100
90	8	0	0

Similar results have been obtained with tobacco mosaic in tobacco juice by various observers. For example, Mulvania<sup>(11)</sup> found that with 10 minutes exposure 80° C. reduced the infectivity not at all, 83° C. to 80 per cent., 85° C. to 50 per cent., 89° C. to 10 per cent., while 90° C. abolished it. (See also Allard<sup>(2)</sup> and McKinney<sup>(9)</sup>.) With tomato mosaic Walker<sup>(16)</sup> found the infectivity destroyed in 10 minutes between 85° C. and 90° C., though on one occasion juice was still infective after 95° C. for the same time.

Filtered juice is still infective after being kept in subdued light at room temperature for one year or more.

*Resistance to Alcohol.* The virus is not destroyed by 90 per cent. alcohol after one hour's contact at room temperature—see Table III. In these determinations absolute alcohol was added to filtered juice in sufficient quantity to give the desired concentration, the volume of juice being the same in all series. After thorough mixing, the vessel was corked and left for one hour at room temperature, and then the mixture was centrifuged for 30 minutes. The supernatant liquid was pipetted off, and tested separately; to the deposit was added distilled water equal in volume to that of the original juice, and after thorough mixing the liquid was inoculated. As Table III shows, the deposit remains fully active even from 90 per cent. alcohol. The supernatant liquid, however, remained slightly active after 70 per cent. alcohol treatment, and still more after 60 per cent. This is not in agreement with Allard's work<sup>(2)</sup>, who with tobacco mosaic found the supernatant inactive after precipitation with 45 per cent. or stronger alcohol. The discrepancy is due probably, not to a difference in method, but to imperfect sedimentation in my experiments, the available centrifuge running at low speed. This is suggested by the variability in the result, as shown in Table III, and also by the fact that when the supernatant was passed through a "L 3" candle, it was quite inactive, even from 50 per cent. alcohol, probably

because the filter removed small flocculi which had not come down in the centrifuge (cf. Olitsky and Boez(13)). Even after 4 days' contact with 60 per cent. alcohol, the virus remained fully active, giving 100 per cent. infection, and repeated washing of the precipitate with 60 per cent. alcohol did not reduce its infectivity.

Walker(16), using tomato mosaic juice, found the precipitate infective after one hour's treatment with alcohol in all concentrations from 33 to 95 per cent. Allard(2), on the other hand, found the precipitate not infective after 1-2 days' contact with 75 or 80 per cent. alcohol, using tobacco mosaic from tobacco; and cucumber mosaic in cucumber juice cannot withstand even 45 per cent. (Doolittle(3)).

Table III.

*Effect of Alcohol.*

Concentration alcohol %	A. After 1 hour's contact and then centrifugalisation.		Supernatant unfiltered		Supernatant filtered	
	Precipitate					
	No. of plants	No. positive	No. of plants	No. positive	No. of plants	No. positive
50	.	.	.	.	8	0
60	8	8	8	6	8	0
60	8	8	8	2	8	0
70	8	8	8	1	.	.
80	8	8	8	0	.	.
90	8	8	.	.	.	.

B. After 4 days' contact with 60 % alcohol : precipitate: 8 plants, 8 positive.

This high resistance to alcohol of certain plant viruses is a remarkable phenomenon, and the only parallel to it to be found in the literature of other virus diseases is in the case of the virus of foot and mouth disease of cattle. But even here the resistance appears to be of a lower order. The English Commission on this disease(4) found that the virus occasionally withstood 60 per cent. alcohol for 18 hours, but never longer in their experience; and the resistance varied with different samples of virus, some being inactivated after 5-6 hours' contact. The supernatant liquid also occasionally remained infective. Olitsky and Boez(13) found that the foot and mouth virus with which they worked resisted 60 per cent. alcohol for 26 hours or more, and that the supernatant liquid was always inactive if care were taken to remove from it, by filtration or otherwise, all undeposited flocculi of precipitate. They believed, however, that this resistance is not a genuine property of the virus. In their view, the virus is really sensitive, but the precipitate (of

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protein or other material present in the liquid) produced by the alcohol protects the virus from its action; and, when the formation of this precipitate is prevented, *e.g.* by modification of the reaction of the liquid by addition of sodium hydrate, the virus is killed in a very short time (a minute or two) and shows no more resistance than *Bacillus coli* or *Staphylococcus*. It seemed desirable to repeat this work with a plant virus.

Preliminary experiments showed that to prevent the formation in tomato juice of visible precipitate on the addition of alcohol to 60 per cent., considerable quantities of NaOH are necessary. Even with 1.0 c.c. of *N/1* NaOH solution and alcohol to 60 per cent. precipitation was usually visible within half-an-hour of adding 5 c.c. of juice, although small in amount and delayed in formation. With 1.3 c.c. up to 2.0 c.c., as a rule, no precipitate appeared, even after some hours. These large amounts of NaOH are, however, themselves toxic to the virus (Table IV).

Table IV.

To the stated volumes of *N/1* NaOH solution in water, was added distilled water to bring the volume to 5 c.c. and after thorough mixing, 5 c.c. of active virus juice were added and well mixed. After 2 hours' contact at room temperature, the mixtures were inoculated to, in each case, six young tomato plants, with the following results:

0.5 c.c. <i>N/1</i> NaOH	100	% positive
1.0 c.c.     "	83	"
1.5 c.c.     "	16.6	"
2.0 c.c.     "	0	"
0            "	100	"

In order, therefore, to prevent precipitation by the alcohol one had to use a quantity of NaOH which already of itself reduced the infectivity of the virus. It seemed, however, that this might make the test of the action of alcohol still more sensitive, and the following experiment was therefore carried out.

To three test-tubes were added 1.3 c.c., 1.6 c.c., and 2.0 c.c. respectively of normal NaOH solution. To these were then added absolute alcohol sufficient to bring the final mixture to 60 per cent., and after mixing well, 5 c.c. virus juice were added, and thoroughly mixed. All the tubes remained free from visible precipitate (and remained clear for 8 hours at least).

Four tubes were then taken, *A, B, C, D*. To *A* were added 1.3 c.c. *N/1* NaOH solution, then 9.45 c.c. absolute alcohol, and these mixed well; then 5 c.c. filtered virus juice were added and thoroughly mixed. After standing at room temperature for 1 hour 50 minutes, during which time no precipitate appeared, the mixture was inoculated to 8 young

Kondine Red plants in the usual way. Of these, two, viz. 25 per cent., developed the disease, both after an incubation period unusually prolonged, viz. 18 and 21 days respectively.

To *B* was added no NaOH, but 1.3 c.c. water instead, and then alcohol and juice as before. After 1 hour's contact, the mixture was centrifuged for 30 minutes, the supernatant liquid removed, and replaced by 5 c.c. water, in which the deposit was well shaken up. This was then inoculated to 8 plants as before, all of which developed the disease, 7 on the 9th day and 1 on the 13th day.

To *C* were added 1.3 c.c. *N/1* NaOH, 9.45 c.c. water (*i.e.* no alcohol), and 5 c.c. virus as before. After 1 hour 45 minutes, the mixture was inoculated to 8 plants. Of these, two, *i.e.* 25 per cent., developed the disease, and again in both cases late, viz. on the 16th and 21st days. The alkali alone, therefore, without alcohol, reduced the infectivity of the virus to the same extent as did the alkali-alcohol mixture.

To *D* were added 10.75 c.c. water, and 5 c.c. virus juice; and after 1½ hours the mixture was inoculated to 8 plants, all of which developed the disease by the 14th day, five of them in 9 days.

It appears then that the presence of alcohol to 60 per cent. in the alkalis mixture did not reduce still further the infectivity of the virus already lowered by the alkali alone, and there is nothing to suggest that in the case of this plant virus, alcohol in the concentration used is really toxic to the virus, and is able, if the formation of a protective precipitate is prevented, to exert its toxic action and destroy the virus.

*Cultivation outside the Plant.* Hitherto all recent attempts to obtain increase of any plant virus outside of living plant tissue have failed, with one exception. Olitsky<sup>(12)</sup>, using no unusual or special technique, inoculated sterile normal tomato juice with the juice of mosaic tomato, subcultured from this into normal juice, and obtained infection even with the 12th subculture. This represented a dilution of  $4/10^{16}$ , far outside any possibly infective dilution of the original inoculum. Several workers have tried to repeat this experiment but without success (Goldsworthy<sup>(5)</sup>, Mulvanian<sup>(10)</sup>, Purdy<sup>(14)</sup>), and no explanation of the difference in result is as yet available. Equal want of success has attended all experiments with the *Aucuba* or yellow type of mosaic, but, since the difference from Olitsky's result may be due to some apparently minor point of technique, one such experiment is given here in some detail in spite of its negative result.

Sterile tomato juice was obtained from young actively growing normal tomato plants by the same method as was used in the

preparation of filtered virus juice, the crude juice in this case being passed through filter-paper, instead of sand and paper-pulp, before being passed through the "L 1" and "L 3" candles. After final filtration it was distributed in 5 c.c. volumes and incubated for 1 week at 27° C. Successive batches of such juice were prepared from time to time, and no juice was used that was more than 3 weeks old. The pH of this normal juice in four successive batches was 5.5, 5.1, 5.5 and 5.5 tested colorimetrically. After incubation, tubes were inoculated in the following manner from young tomato plants showing recent and well-marked signs of the Aucuba disease. Petioles, or young stems, were cut across with a sterile scalpel, seared at the end with a red hot knife, capillary tubes inserted through the seared surface and small quantities of juice sucked up and transferred to tubes of the medium. In all, 21 tubes were so inoculated, constituting the 1st subculture, and were then incubated at 27° C. After 5 days, from each of these tubes 0.1 c.c. was transferred to a tube of fresh medium (a separate pipette being used for each tube); this constituted the 2nd subculture. The process was repeated every 5 days, giving the 5-day series of cultures. Similarly, every 10, 15 and 25 days, subcultures were made, giving the 10-day etc. series. There were therefore 4 series of subcultures, differing in the periods of incubation. Any tube showing obvious contamination was rejected, but this rarely occurred. From time to time tests of the subcultures were made by inoculation to plants. For this purpose, from each of all the tubes of a subculture in any one series 0.2 c.c. was withdrawn as a sample, the samples mixed together, and the mixture inoculated to young tomato plants in active growth. Inoculation was made by needle prick, at least 80 punctures per plant in 4 leaves, and further, a small pledget of cotton wool soaked in the juice was inserted into an incision in the stem. The plants were held for at least 5 weeks, and examined regularly. The batches of uninoculated medium were tested in the same way, always on 6 to 8 plants.

The results of all inoculations made up to the 4th subculture are shown in Table V. The amount of the original inoculum of infected material could not be exactly measured, and varied a little in every tube with the quantity of juice taken up in the capillary, which also took up small pieces of tissue. It was estimated to be about 0.01, and not to exceed 0.05 c.c. in any tube. Taking the larger figure as an outside estimate the dilution of the inoculum was in the 1st, 2nd, 3rd and 4th subcultures,  $1 \times 10^2$ ,  $5 \times 10^3$ ,  $25 \times 10^4$  and  $125 \times 10^5$  respectively. The 3rd subculture, then, represents a dilution of the original inoculum of

Table V.

Series (days)	Subculture	No. of plants	No. positive	% positive
5	1st	4	2	50
	2nd	6	1	16.6
	3rd	8	1	12.5
	4th	10	0	0
10	3rd	6	2	33.3*
	4th	8	0	0
15	4th	8	0	0
25	4th	8	0	0

\* This figure is unreliable, since the test of the batch of medium used for this subculture gave one positive result.

1 in 250,000, a dilution still possibly infective; but the 4th subculture, being a dilution of 1 in 12,000,000, is outside the range of still infective dilution. As is shown in Table V, in no case was infection got with the 4th subculture (nor in several examinations of later subcultures); but in one at least of the series the 3rd subculture was still slightly infective. No evidence, therefore, was obtained of multiplication of the virus.

This experiment was repeated the next season, using as normal medium a juice differently prepared. Here the tissue was ground up in an apparatus devised by W. A. Roach<sup>(15)</sup>, and so finely that no intact cells could be detected under the microscope; the liquid was then passed through a "L 1" and then a "L 3" candle. The preceding experiment was then repeated, using only one series, viz. 7 days' incubation, and the original inoculum consisting of 0.1 c.c. of filtered virus juice, which was proved to be very active. Eight plants were inoculated with each subculture: the 1st subculture gave 7 positive, the 2nd 2, the 3rd, 4th and 5th, none.

It was again repeated in 1927 in this laboratory by Dr H. H. Storey on the lines of the first experiment described above, using a 7-8 days series, and inoculating with active filtered juice. In this also no evidence of multiplication was obtained. A number of modifications of different kinds has been tried, but all have failed.

Our experience, therefore, has been the same as that of all other workers, with the exception of Olitsky. There is, however, a possible explanation of our failure, which has not, so far as we know, been tested in the case of plant viruses. It may be, that for successful inoculation two factors are necessary, the virus itself and an accessory, non-multiplying factor, of which neither alone is sufficient to produce the disease but the two together are capable of causing infection. This, according to Gye<sup>(6)</sup>, is true of the filterable chicken sarcomas of Rous.

It might, therefore, be the case that growth of the virus did occur in our subcultures, but its presence was not detected on inoculation owing to the loss of the accessory factor either through the high dilution involved in the subculturing or through ageing or deterioration. We have not so far succeeded in demonstrating the existence of such an accessory factor, and only one experiment, carried out here by Dr H. H. Storey, will be mentioned. Virus juice was precipitated by 60 per cent. alcohol, and the supernatant liquid filtered. This supernatant liquid is itself, as has been shown above (p. 160), incapable of producing the disease, nor, when it was added to the 4th subculture of virus in normal juice, did the mixture become active, either with or without preliminary evaporation of the alcohol from the supernatant liquid.

I have pleasure in thanking Miss M. M. Browne for her assistance in the growth and care of the many plants required in these experiments.

#### SUMMARY.

A description is given of a mosaic disease produced in tomato by a virus, possibly identical with Johnson's Tobacco Virus 6, which differs from that of ordinary tomato mosaic in the brilliance and intensity of its leaf-symptoms, but in other respects is indistinguishable from it by the characters investigated.

The filtered juice of infected plants transmits the disease in dilutions in water up to 1 in 10,000, retains its activity for a year or more at room temperature, and withstands heating for 10 minutes at 80° C. but is inactivated at 90° C.

It is not inactivated by alcohol up to 90 per cent. The virus comes down with the precipitate, and is not destroyed when the formation of precipitate is prevented by the addition of NaOH.

Attempts at cultivation of the virus outside the living plant are described; all were unsuccessful. The methods employed in filtration, inoculation, etc. are given in detail.

#### REFERENCES.

- (1) ALLARD, H. A. (1915). *Journ. Agric. Res.* III, 295.
- (2) — (1916). *Ibid.* VI, 649.
- (3) DOOLITTLE, S. P. (1920). *U.S. Dept. Agric., Bull.* 879.
- (4) *Foot and Mouth Disease Research Commission, First Progress Report.* London, 1925.
- (5) GOLDSWORTHY (1926). *Phytopath.* XVI, 873.
- (6) GYE, W. E. (1925). *Lancel.* CCIX, 109.
- (7) HOGGAN, I. (1927). *Journ. Agric. Res.* XXXV, 651.



Fig. 1.



Fig. 2.



- (8) JOHNSON, J. (1927). *Wisconsin Agric. Exp. Sta. Res. Bull.* LXXVI.
- (9) MCKINNEY, H. H. (1927). *Journ. Agric. Res.* XXXV, 1.
- (10) MULVANIA, M. (1925). *Science*, n.s. LXII, 37.
- (11) — (1926). *Phytopath.* XVI, 853.
- (12) OLITSKY, P. K. (1925). *Journ. Exp. Med.* XLI, 129.
- (13) — and BOEZ, J. (1927). *Ibid.* XLV, 815.
- (14) PURDY, H. A. (1926). *Bot. Gaz.* LXXXI, 210.
- (15) ROACH, W. A. (1925). *Biochem. Journ.* XIX, 783.
- (16) WALKER, M. (1926). *Phytopath.* XVI, 431.

### DESCRIPTION OF PLATE IX.

Fig. 1. Typical leaves of tomato infected with Aucuba or yellow mosaic.

Fig. 2. Leaf of tomato similarly infected, showing yellowing of the veins.

Photographs taken by V. Stansfield.

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## THE TRANSMISSION OF POTATO MOSAIC TO TOMATO

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(With Plates XXIV-XXVI.)

### INTRODUCTION.

TRANSFERENCE of virus disease in potato to other potatoes or other hosts is complicated by two facts which were unknown to, or imperfectly appreciated by, the earlier workers, and invalidate some of their conclusions. The first of these is the existence of carriers. A given variety of potato may show no sign of disease, growing healthily and giving a good yield, and yet it may be carrying in a masked condition a virus disease, *e.g.* streak, which will produce the most marked symptoms in another variety on transference to it. Further, a particular potato may be obviously infected with one disease, *e.g.* mosaic, and at the same time be carrying a second disease of which it shows no symptoms, but on inoculation to another variety, intolerant of this second disease, the new host may develop the signs of the second disease, sometimes in the most unexpected form, and show little or no sign of the first disease which was obvious in the original host. As Atanasoff<sup>(1)</sup> says, one of the common difficulties in potato virus work is the appearance of an entirely different disease in the artificially infected plants. Unless it has been shown by careful preliminary tests that concealed disease is not present, the results of inoculation into another host may be most misleading.

The second complication is the possibility, suggested by Johnson<sup>(2)</sup>, that material from apparently perfectly normal potatoes may have the property on inoculation into tobacco of evoking a virus disease in the new host. Whether this is simply a special case of the carrier will be referred to later on, but many of the aberrant results recorded in the literature are certainly to be attributed to this phenomenon.

Attempts to transmit potato virus disease to other hosts have been reported by several observers. Quanjer<sup>(4)</sup> failed to transmit potato "mosaic" to tobacco by grafting, and Schultz and Folsom<sup>(5)</sup> also failed

to transmit potato mosaic to tobacco either by leaf-mutilation or by spinach-aphids. There seems, indeed, to be no definite record of successful transmission of any potato virus disease to tobacco, before the experiments of K. M. Smith<sup>(6)</sup>, who produced ring-spot by leaf-mutilation inoculation of material from Arran Victory potatoes affected with mosaic. Transference to tomato, however, has been recorded in a number of cases. Quanjér<sup>(4)</sup> transmitted potato "mosaic" by grafting, though not with all varieties of potato, and he states that crinkle, aucuba and leaf-roll are all transmissible to tomato, though in leaf-roll no signs may appear in the tomato and regrafting back to healthy potato may be necessary to demonstrate that transmission has occurred. Schultz and Folsom<sup>(5)</sup> by leaf-mutilation also succeeded in transmitting mild mosaic and rugose mosaic to tomato, getting signs in the new host similar to those in the original potato. Inoculation with streak material also produced disease in tomato, though this disease might be more like rugose mosaic than streak. Vanterpool<sup>(7)</sup>, Fernow<sup>(8)</sup>, Berkeley<sup>(9)</sup> and others record the development of a peculiar mottling in tomato on inoculation with potato virus material of different kinds, the appearance in the tomato usually bearing no resemblance to that in the potato and sometimes developing even when the inoculum was derived from potatoes showing no signs of disease. Some at least of these results may be attributed to the phenomenon described by Johnson who indeed says<sup>(3)</sup> that "the host range of potato viruses is apparently restricted to the potato." Even K. M. Smith's transmission of mosaic to tobacco might be included in this group, were it not for his failure to obtain any such disease with material from potatoes of the same variety proved to be free from virus.

The experiments described in this paper deal chiefly with the transmission of mild mosaic of potato to tomato and with the characters of the disease so produced. No disease was obtained in tomato on inoculating with material from healthy potatoes, but a definite and characteristic disease was regularly produced on inoculation with material from potatoes infected with mild mosaic.

#### METHODS.

The method of inoculation was the same in all cases. Leaves were taken from the potato, minced and thoroughly ground in a mortar, then 3 c.c. of distilled water for each 1 gm. of tissue were gradually added with renewed grinding, and the resulting liquid was inoculated to at least six, usually eight, tomatoes. The liquid was dropped on a leaflet supported on a wooden slip and the leaflet scratched with a needle

through the liquid. Forty to fifty scratches were made per leaflet, and four leaflets were inoculated per plant. This gave an inoculation which was perhaps unnecessarily heavy, but it was intended to ensure that in the inoculation with normal material a sufficient dose should be given. The tomato plants were always quite young, growing rapidly and with only three to four leaves showing leaflets large enough for inoculation. They were grown in insect-proof cages until used, and after inoculation were usually returned to the cages, though sometimes, owing to lack of available space, the inoculated plants were kept on the bench in the glass-house, which was fumigated regularly. All plants were grown at temperatures over 50° F. The variety of tomato chiefly used was Kondine Red; but other varieties, such as Blaby, were also used, without difference in the results.

#### NORMAL POTATOES.

Leaves from normal potatoes of nine different varieties have been so inoculated, viz. Majestic, Arran Chief, Arran Victory, Epicure, Sharpe's Express, Great Scot, President, Abundance and King Edward. I am indebted to Dr Salaman, Dr G. F. Pethybridge and Mr H. Bryan for much of this material. The greatest care has been taken to ensure that these potatoes did not harbour concealed virus. In a number of cases the stock from which foliage was taken had been repeatedly grafted by Dr Salaman with potatoes very susceptible to streak and mosaic, such as President and Arran Victory, without producing any disease in them; and I believe that all were in fact free from virus disease. The inoculated plants were held for at least four weeks, in some cases for six weeks or more. In no case was any disease produced in the tomatoes. In several instances leaves were taken from the inoculated tomatoes and again inoculated into a second generation of six to eight young tomatoes without producing any symptoms in them. I have had no case where a potato, which had been shown to be normal on preliminary testing, produced disease in tomato.

It seems clear that in this country and with these varieties of potato, potato protoplasm as such does not produce virus disease in tomato, and it is reasonable to suppose that the same would hold good of other varieties as well. One example may be given of the possible value of inoculation into tomato as a guide to mosaic infection in potatoes. A number of potatoes of the variety Kerr's Pink, which were apparently normal, had been grown by Dr Salaman. They had not yet been tested by grafting, and presented some very slight discoloration of the foliage,

which aroused suspicion. With his consent leaves of these plants were taken and inoculated to tomatoes, in which they produced characteristic signs of disease within fourteen days; and later, the original Kerr's Pink plants, from which these leaves had been taken, developed definite symptoms of mosaic disease.

#### MOSAIC POTATOES.

On the other hand, leaves from mosaic potatoes have invariably produced in tomato a definite disease, and up to the present all the inoculated tomatoes have shown it. The following varieties of potatoes infected with mosaic have been used: Majestic, Arran Chief, Arran Victory, Up-to-Date and Kerr's Pink. All have exhibited the same general symptoms, of which a description is given later. Three of these varieties were used in the normal series, when they produced no disease in tomato, and it is reasonable to conclude that the symptoms produced in the tomatoes were due to the mosaic present in the potatoes. Normal plants of Up-to-Date and Kerr's Pink have not yet been procured. The Up-to-Date potatoes used presented certain features of interest. As is well known, the Up-to-Date variety is a persistent carrier of streak, of whose presence it shows no signs, but when grafted with a susceptible variety such as Arran Victory, the latter goes down with an extreme form of streak disease. Dr Kenneth Smith gave me some shoots of Up-to-Date which had no visible signs of mosaic at the time, but the plant was known to be infected with mosaic. He also gave me some shoots of Arran Victory which had been grafted with the same Up-to-Date plant, and showed the characteristic lesions of very virulent streak. Leaves from the Up-to-Date were inoculated to tomato and produced the usual disease, but leaves from the streaked Arran Victory inoculated at the same time to eight tomatoes of the same batch and kept in the same chamber of the glass-house produced no symptoms at all. No conclusion, of course, can be drawn from a single instance, but this result would suggest that streak may not be transmissible to tomato, at least not by this method of inoculation. It is noticeable that the mosaic present in the Up-to-Date and able to produce lesions in tomato from that variety was not present in the Arran Victory in such a form, or perhaps in such a quantity, as to produce any perceptible effect in the tomato, though presumably the mosaic must have had the chance of passing with the streak to the Arran Victory through the graft. Arran Victory is, as already mentioned, susceptible to mosaic and can transmit it to tomato in the absence of streak.

Other examples have been found where potatoes showing mosaic produced the characteristic lesions in tomato, but they are not detailed in the present paper, since opportunity has as yet been lacking to make certain that they contained no other virus disease than the visible mosaic.

#### CHARACTERS OF THE DISEASE.

The disease as it appears in the tomato may take either of two distinct forms, which, however, may occur concurrently. As a rule, the first signs appear within fourteen days in the form of small necrotic spots. These come quite suddenly, often first in leaflets of the same leaf as an inoculated leaflet, but also often first on the leaf next above the inoculated leaves. They are usually isolated at first (Plate XXIV, fig. 1), but rapidly increase in number and may eventually coalesce to form larger necrotic areas. This condition may remain the only symptom for many days, but usually some mottling develops, either on leaflets already spotted or on other leaves. The second form in which the disease may appear is a mottle, and on the whole this is more common than the pure necrotic spot type. This also appears first on leaflets near the inoculated leaflets or on the leaf next above the highest inoculated leaf, usually first near the tip of the leaflet, and spreads upwards in the plant as it grows. The type of mottle is a spotting of paler green, sometimes rather fine in grain, sometimes coarser (Plate XXIV, fig. 2) and the spots tend to coalesce, so that the whole area of leaf affected becomes irregularly chlorotic with spots of still paler colour visible in it. The spots may show a tendency to form small rings of pale green or yellow enclosing a darker centre, but this ring formation is not so well marked in tomato as in some other hosts. In many cases this mottling is the only obvious symptom throughout the life of the plant, no necrosis developing at any time, but in most of these plants a few necrotic spots develop, one or two in every leaf, similar to those seen in the first type. In the first transfer from potato there is a distinct tendency for the mottling to fade and become much less obvious after a time, and a plant which had well-marked signs three weeks after inoculation may show very little a month later. After several transfers in tomato this fading rarely occurs.

There is no necrotic streaking of the stems or petioles at any time. In this it differs definitely from the streak or stripe of tomatoes common in glass-houses, to which disease the necrotic spot type bears a considerable resemblance. The distribution of the signs is also unlike that of tomato streak. In the disease here described the uppermost younger leaves usually remain free from signs throughout. Even after many generations

in tomato, the upper part of the plant seems quite normal, and the plant looks as if it were growing out of the disease. This, however, is not the case: as the young leaves in turn grow older and larger, they also develop the mottle or spots, and the symptoms spread gradually up the plant. In streak, on the other hand, it is common to find the youngest leaves streaked and spotted even at the very tip of the plant, and in the mottle form of that disease the mottling occurs also on the youngest leaves. The mottle in streak, moreover, is of a different type, larger and more blotched, and more like ordinary tomato mosaic.

Potato mosaic is not very virulent for tomato. The infected plants, though their growth is less than that of control plants, develop well and produce flowers and fruit. When combined with the yellow or aucuba mosaic of tomato, however, it causes a very severe disease, which has all the characters of true streak (Henderson Smith<sup>(10)</sup> and cf. Vanterpool<sup>(7)</sup>, Dickson<sup>(11)</sup>). Even after years of propagation in tomato without return to the potato, it regularly produces this virulent disease when associated with the yellow mosaic, whether the two are inoculated simultaneously, or either is added to a plant already infected with the other. When the juice of a plant so streaked is treated with 90 per cent. alcohol for one hour, the potato mosaic factor of the combination is inactivated (see *infra*, p. 525) and the treated juice gives a pure yellow mosaic infection.

As already stated, up to the present time every tomato inoculated directly from mosaic potato has shown signs of infection; and in the very numerous subsequent transfers made from tomato to tomato, whether with filtered or unfiltered juice, failures to obtain transmission in all inoculated plants have been rare, provided the plants are growing well. The effects in the tomato, however, are not uniform. Besides the fact that some plants may show mottling almost exclusively and some only spot-necrosis, the signs vary considerably in intensity in different plants. In some cases they may be so slight that they might easily be missed, in others they are strikingly obvious. This variability makes it difficult to determine whether there is any difference in the several strains of potato mosaic we have used, or whether the variety of potato in which the mosaic is found affects its virulence for tomato. With Arran Victory mosaic all the tomato plants inoculated developed pronounced spot-necrosis with scarcely any mottle (which, however, was well marked in later transfers from tomato to tomato), while with Up-to-Date mosaic the tomatoes all showed marked mottling at first and necrotic spots only later. There has been no difference in symptoms so constant or definite as to justify a distinction between the mosaics used. With Majestic mosaic the inoculation produced only a slight mottling in the first

generation of tomato, a very definite mottle in the second, and in the third conspicuous spot necrosis with much mottling. This apparent increase of virulence was maintained in transfers for over a year, but recent transfers have given very little spotting.

When brought back from tomato into normal potato again, the original disease is reproduced in the latter in a more intense form: a very obvious mosaic, which develops in two or three weeks. We have seen no necrosis of the leaves or stems of the inoculated potatoes. Even after long propagation in tomato, inoculation into potato still produces typical mosaic in the latter. Cuttings were taken from healthy plants of President and Arran Chief, grown in sand, and, later, in soil, and when of suitable size, were inoculated in the usual way with the mosaic originally obtained from Majestic potato and maintained in tomato for two years. Intense mosaic developed in all the inoculated plants in fifteen days, the controls remaining healthy.

From tomato the potato mosaic is readily transmissible by leaf inoculation to other solanaceous plants. Of fifteen different plants inoculated, two only, viz. *Solanum melogena esculentum* and *Physalis francheti*, developed no symptoms. In the others leaf symptoms appeared within three weeks in every case, viz. in *Datura stramonium*, *Nicotiana tabacum* (White Burley), *N. affinis*, *N. Sanderae*, *Solanum nigrum*, *S. dulcamara*, *S. villosum*, *S. nodiflorum*, *Hyoscyamus niger*, *Nicandra physaloides*, *Petunia violacea*, *Capsicum annuum*, *Salpingoglossis sinuata*. These symptoms are quite unlike those produced by the yellow mosaic, which is transmissible to all of these hosts except *S. dulcamara*, *Physalis francheti* and *Datura stramonium* (though in the last, inoculation in the stem produces a localised necrosis without leaf signs). The symptoms have a general resemblance to one another in most cases, except in *Nicandra physaloides* where they appear as rather large (about 4 mm.) yellowish spots or blotches on the upper leaves, and smaller black necrotic spots on the lower leaves which show also a general chlorotic yellowing. The resemblance is not very close, but there is a tendency to form small rings in many of the hosts. This is well marked in *Hyoscyamus niger* (Plate XXV, fig. 3) and *Datura stramonium* (Plate XXV, fig. 4). The rings are usually small, about 1 to 2 mm. in diameter, much smaller than those figured by K. M. Smith<sup>(6)</sup> as occurring in a different variety of tobacco, and probably smaller than the ring-spot described by Johnson<sup>(2)</sup>. They appear as of a paler green with dark green centre, and tend to turn into spots, the centre also becoming chlorotic and occasionally even necrotic. In tobacco (Plate XXVI, fig. 5) the pattern is very like the spot-necrosis disease of Johnson, figured by Hoggan<sup>(12)</sup>.

## CHARACTERS OF THE VIRUS.

The characters of the virus of potato mosaic outside the plant have been studied chiefly in juice prepared from infected tomatoes by methods fully described elsewhere<sup>(10)</sup>. It is filtrable, and after filtration through first an L. 1 and then an L. 3 Pasteur Chamberland candle produces infection when diluted 1 in 1000 with distilled water (100 per cent. of inoculated plants) and 1 in 10,000 (40 per cent.), but not when diluted 1 in 100,000. The pH of the filtered juice varied in different samples from 5.9 to 6.4. After simple clarification of the crude extract by passage through one layer of filter-paper, the juice still failed to infect when diluted 1 in 100,000 but gave incomplete infection in 1 in 10,000.

The virus is less resistant to heat than either the yellow mosaic or ordinary tobacco mosaic, being inactivated in ten minutes at 80° C. in all cases, and sometimes at 70° C. (see Table I). In potato juice the action of heat is similar. Juice was extracted from President potatoes infected with the Majestic mosaic, and filtered through candles in the usual way. After heating for ten minutes at 50° C., this filtered juice produced 100 per cent. infection; at 60° C., 100 per cent.; at 70° C., 17 per cent.; at 80° C., no infection.

Table I.

*Effect of Heat on the Virus of Potato Mosaic.*

Temperature	Majestic virus (a)	Up-to-Date virus (a)	Arran Victory virus (a, b)	Arran Chief virus (b)	Kerr's Pink virus (b)
50° C.	100	100	100	100	83.3
60° C.	100	100	100	100	80
70° C.	20	0	0	50	0
80° C.	0	0	0	0	0
90° C.	0	0	—	0	0
Unheated	100	100	100	100	100

The figures denote the percentage of tomato plants which showed infection; six to eight plants were inoculated in every case.

(a) Test made with juice filtered through candles.

(b) Test made with juice passed through one layer only of filter-paper.

To alcohol the virus is also less resistant than the yellow mosaic, being inactivated by 90 per cent. after one hour's exposure, and sometimes by 80 per cent. (Table II). The filtered potato juice (Majestic mosaic in President), after one hour's exposure to 50 per cent. alcohol, produced 100 per cent. infection; to 60 per cent., 43 per cent.; to 70 per

cent., 29 per cent.; to 80 per cent., 57 per cent.; to 90 per cent., no infection. Here again the Majestic and Arran Chief strains showed higher resistance than Up-to-Date.

Table II.

*Effect of Alcohol on the Virus of Potato Mosaic.*

Alcohol %	Majestic virus (b)	Up-to-Date virus (a)	Arran Chief virus (b)
50	—	100	—
60	100	85.7	100
70	100	42.8	100
80	37.5	0	50
90	0	0	0
Untreated	100	100	100

For explanation of figures and letters, see Table I.

A similar difference was found in regard to ageing. In filtered juice (tomato) Majestic mosaic remains infective for five and a half months, the longest period yet tested. Up-to-Date mosaic, however, was found to be inactive after 12 weeks, the filtered juice having been kept in dull light in paraffin-stoppered tubes.

The virus in filtered juice withstood 20 per cent. chloroform for four hours at 27° C., and the dyes Auramine O and Meldola Blue, diluted 1 in 2000, for two hours at 27°. Acriflavin, 1 in 1000 for the same time at 27° C., did not wholly destroy it, and 1 in 5000 did not affect it perceptibly. Meldola Blue, 1 in 500, did not completely inactivate it. Formalin, 1 in 500 for two hours at 27°, apparently killed it, but 1 in 1500 did not reduce its infectivity.

## DISCUSSION.

The marked difference in appearance of the two types of symptoms, the spot-necrosis and the mottle, suggests the possibility that these potato mosaics are made up of a mixture of two viruses. This may be the case, but we incline to the view that there is only one virus present and the two types of symptoms indicate a difference in reaction of individual plants. In the alcohol and heat series no differentiation occurred—*e.g.* after treatment with 60 per cent. alcohol the juice might give a pure spot-necrosis reaction in one plant and a pure mottle in another plant of the same batch, inoculated at the same time with the same material and kept under the same conditions. The type of symptom is affected to some extent by external conditions—*e.g.* growing the

inoculated plants at temperatures over 70° F. markedly reduced the necrotic spotting and favoured the mottling.

The disease here described bears a very close resemblance to the spot-necrosis disease described by Johnson(2) as obtained in tobacco by inoculation with the foliage of normal potatoes. The character of the symptoms, the occurrence of both the mottle and the spot-necrosis types, the distribution of symptoms and the tendency of the plant to grow out of the disease, the apparent increase of virulence in some cases (not in all) on continued transference in the new host, the thermal death-point, are all so closely alike in the two diseases that it is difficult not to believe that they are very closely related, if not identical. We have, however, never produced this disease with the foliage of normal potatoes (cf. K. M. Smith) and always produced it with the foliage of mosaic potatoes. Whether the two diseases are the same or not, it is evident that the normal potato in the United States differs from the normal potato in this country: unless, indeed, the potatoes accepted by Johnson as normal were not, in spite of the precautions he took, free from a form of suppressed mosaic. It is doubtful whether it is possible by inspection alone, however careful and regular and long-continued, to determine whether a potato is or is not infected with mosaic.

There are certain points in which the disease here described differs from the spot-necrosis of Johnson. His virus is inactivated by one hour's exposure to 50 per cent. alcohol: ours is not inactivated by 70 per cent. for the same time and not in every case by 80 per cent. His virus is inactivated by simple keeping or ageing in most cases in less than twenty days and sometimes in ten days: the virus (Majestic) here described remained infective for more than five months in tomato juice. His virus therefore differs from those described in this paper in its less resistance to ageing and to alcohol. Similar differences occur among the viruses we have worked with. These fall into two groups, the one represented by Majestic mosaic, not wholly inactivated at 70° C. nor by 80 per cent. alcohol and remaining active in filtered juice kept for five months, the other by Up-to-Date mosaic, inactivated at 70° C. and by 80 per cent. alcohol and by simple ageing for 12 weeks (possibly sooner). There would seem to be a series of viruses, all producing similar symptoms and all closely related to one another but differing in susceptibility. At the one extreme we have the virus of Johnson, inactivated by 50 per cent. alcohol and by simple keeping for two or three weeks; at the other extreme the Majestic virus, much more resistant to both alcohol and ageing, while the Up-to-Date virus has an intermediate position.

Further work is necessary before we can conclude with certainty that these differences in susceptibility indicate a real and constant difference in the viruses themselves; but they undoubtedly suggest that there exist strains or varieties of the virus of potato mosaic, and that mild mosaic in the potato may be due to one or more of several allied virus strains, as yet indistinguishable by the symptoms they produce.

I have pleasure in thanking Miss M. M. Browne for the care and skill with which she has grown the many plants required in these experiments.

#### SUMMARY.

Inoculation by leaf-mutilation with the foliage of normal potatoes produced no disease in tomato. Nine varieties of potato were tested.

Similar inoculation with foliage of mosaic potatoes produced a characteristic disease in tomato. Five varieties of potato were used, of which three had been tested in the experiments with normal foliage.

The characters of the disease are described. It is transmissible back to potato again and to other solanaceous plants. The virus is filterable, is still infectious after high dilution of the extracted juice, and remains active on keeping for several months. It is less resistant to heat and alcohol than ordinary tobacco mosaic.

The disease resembles closely the spot-necrosis disease described by Johnson as obtained by inoculation of tobacco with foliage of normal potatoes, the chief difference being the greater resistance of the potato mosaics here described.

It is probable that there exist several strains, differing in resistance, of the virus causing mosaic in the potato.

#### REFERENCES.

- (1) ATANASOFF, D. (1925). *Phytopath.* xv, 171.
- (2) JOHNSON, J. (1925). *Wisconsin Agr. Expt. Sta. Res. Bull.* LXIII.
- (3) — (1927). *Ibid.* LXXVI.
- (4) QUANJER, H. M. (1920). *Phytopath.* x, 35.
- (5) SCHULTZ, E. S. and FOLSOM, D. (1923). *Journ. Agr. Res.* xxv, 43.
- (6) SMITH, K. M. (1928). *Nature*, CXXI, 904.
- (7) VANTERPOOL, T. C. (1926). *Phytopath.* xvi, 311.
- (8) FERNOW, K. H. (1925). *Cornell Univ. Agr. Exp. Sta. Mem.* xcvi.
- (9) BERKELEY, G. H. (1927). *Scientific Agriculture (Canada)*, vii, 210.
- (10) HENDERSON SMITH, J. (1928). *Ann. App. Biol.* xv, 155.
- (11) DICKSON, B. T. (1925). *Science*, LXII, 398.
- (12) HOGGAN, I. A. (1927). *Journ. Agr. Res.* xxxv, 651.

DESCRIPTION OF PLATES XXIV—XXVI

PLATE XXIV.

- Fig. 1. Leaves from tomato inoculated with potato mosaic, showing the spot-necrosis type of symptom.  
Fig. 2. Leaves from tomato inoculated with potato mosaic, showing the mottle type of symptom.

PLATE XXV.

- Fig. 3. Leaf of *Hyoscyamus niger*, inoculated with potato mosaic. Size of leaf  $5\frac{1}{4}$  in.  
Fig. 4. Leaf of *Datura stramonium*, inoculated with potato mosaic. Size of leaf  $5\frac{1}{4}$  in.

PLATE XXVI.

- Fig. 5. Leaf of tobacco (var. White Burley), inoculated with potato mosaic. Size of leaf  $9\frac{1}{2}$  in.

Photographs by V. Stansfield.

(Received May 8th, 1928.)



Fig. 2.



Fig. 1.



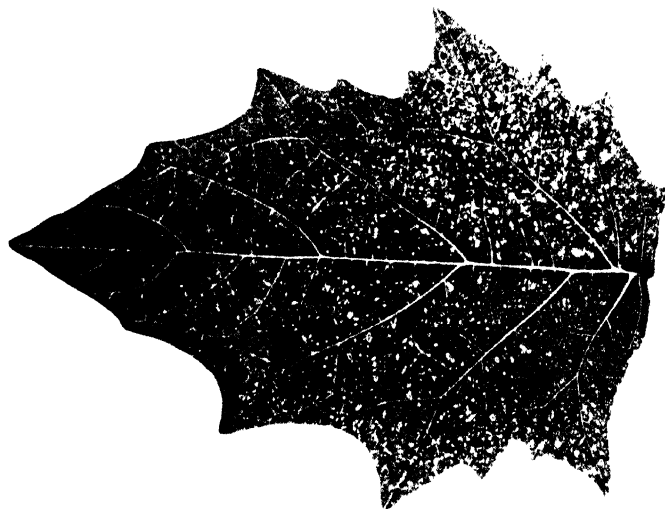


Fig. 4.



Fig. 3.



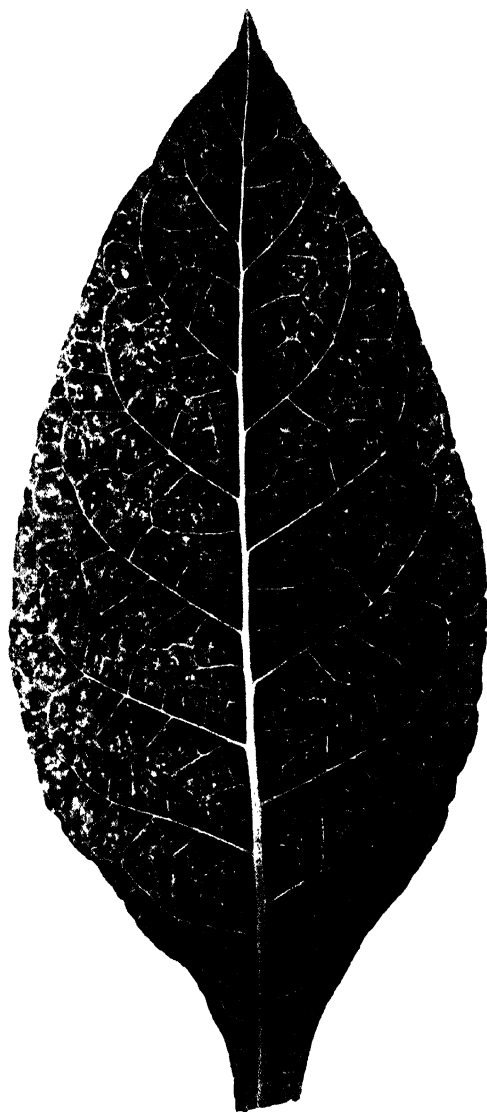


Fig. 5.

HENDERSON SMITH.—THE TRANSMISSION OF POTATO MOSAIC TO TOMATO (pp. 517-528).



## THE INFLUENCE OF ENVIRONMENTAL CONDITIONS ON THE DEVELOPMENT OF THE ANGULAR LEAF-SPOT DISEASE OF COTTON

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(With Plate XVIII and 3 Text-figures.)

THE bacterial disease of cotton caused by *Bacterium malvacearum* E. F. S. (*Pseudomonas malvacearum*), first noted by Atkinson<sup>(1)</sup> in 1891 and described in detail by Erwin F. Smith<sup>(2, 3)</sup> in 1901 and 1920, has rapidly assumed serious importance. The disease appears to be universally distributed throughout the cotton-growing countries of the world and occurs to some extent at least on all species of *Gossypium*, though some degree of varietal resistance has been reported. The disease presents three distinct manifestations according to the part of the plant attacked: (1) water-soaked spots on the leaves passing later into angular lesions delimited by the veins—the “angular leaf-spot,” (2) blackish lesions an inch or more in length on the young stems, sometimes resulting in girdling of the stem and death of the plant, the so-called “black-arm” disease, and (3) blackish spots on the immature bolls, up to a centimetre or even more in diameter, sunken and rounded in outline—the “bacterial boll-rot.” All three of these forms of the disease have been obtained under glasshouse conditions by the writer by means of varied methods of artificial inoculation, but the present paper deals only with a series of experiments carried out on the conditions governing the development of the angular leaf-spot manifestation on young plants.

Atkinson<sup>(4)</sup> in 1892 suggested that bad infections might be due to climatic conditions unfavourable to the cotton plant or conversely conditions favourable to the organism. Erwin Smith<sup>(3)</sup> showed that infection was stomatal, and Rolfs<sup>(5)</sup> concluded that in absence of moisture infection could not occur. Snowden<sup>(6)</sup> states that the disease is checked by dry weather. Nakata *et al.*<sup>(7)</sup> decided that in Korea the occurrence of the disease was largely governed by the environmental conditions, and stated that lack of potash in the soil and a wet season were two factors which especially favoured the development of the disease.

In 1927 the writer decided to carry out experiments on the artificial inoculation of cotton plants with the organism under controlled environmental conditions. After some early unsuccessful attempts, the following apparatus was designed and proved satisfactory.

*Description of infection chamber.*

The apparatus (Fig. 1) is essentially a double-walled infection chamber suitably insulated against heat-loss and provided with means for maintaining the air temperature and humidity within it at any

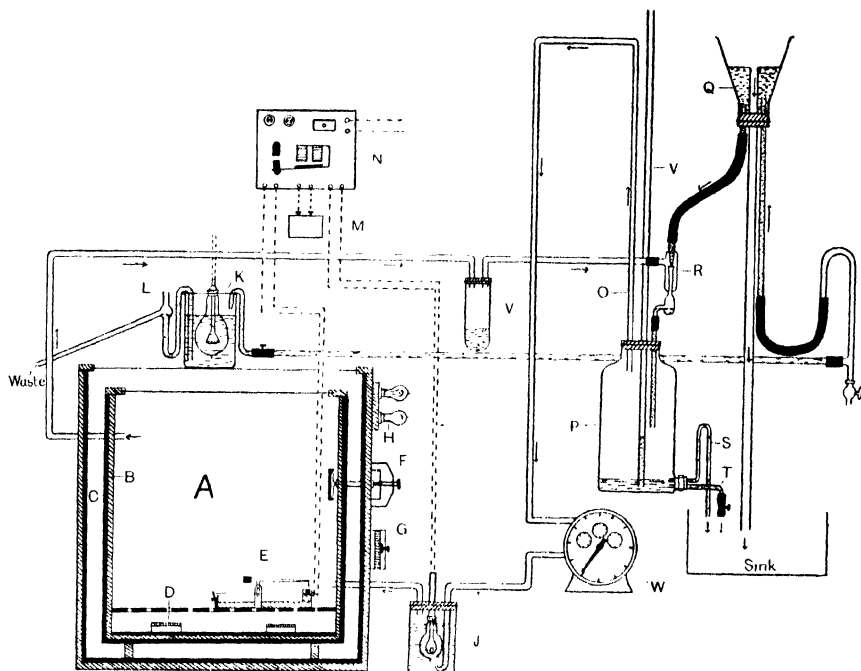


Fig. 1. *A*, infection chamber; *B*, wooden walls (cross hatched); *C*, asbestos linings; *D*, heating units; *E*, hygrometer; *F*, temperature switch; *G*, variable resistance; *H*, resistance lamps; *J*, conditioning vessel; *K*, lamp for illumination; *L*, constant-level overflow; *M*, battery; *N*, relay; *O*, air tube; *P*, aspirator; *Q*, glass funnel; *R*, filter pump; *S*, regulating outflow; *T*, constant outflow; *V*, trap for condensed water; *W*, air meter.

required degree. The chamber is made by fixing a wooden box (in this case a tea chest 20 in. × 20 in. floor space) on blocks within a larger packing-case so as to leave a dead-air space of about two inches all round between the boxes. The inner walls and floor of the outer case and the outside of the inner chest are covered with "Uralite" asbestos-

board as lagging, and each box is provided with a hinged glass lid. On the floor of the inner case is another square of asbestos board carrying the four heating units, which in this case were small wire-wound mica "strip-heaters" as used in small electric incubators. These are held in position by brass clips screwed to the asbestos, and the electrical connections are made to the clips. An inch above the heating units is a false bottom also made of "Uralite" and pierced by a large number of holes; this serves to distribute the heat more uniformly. On the front wall of the chamber is fixed a Hearson Electric Temperature Switch (*F*), the

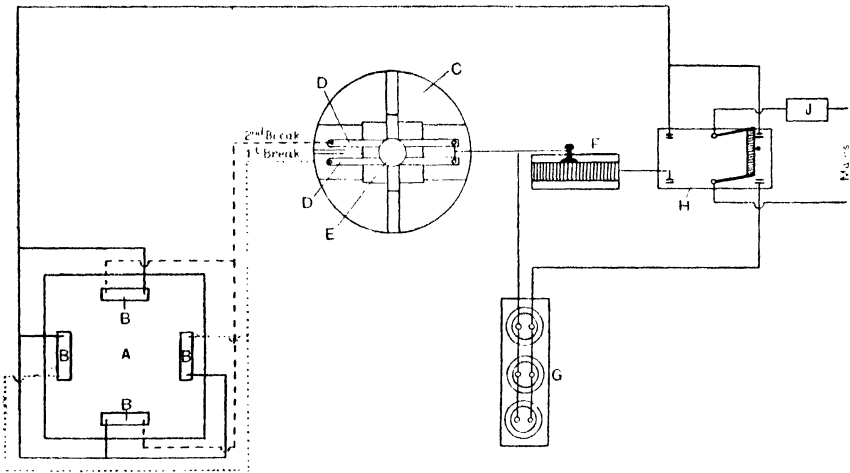


Fig. 2. *A*, asbestos bottom of infection chamber; *B*, heating units; *C*, temperature switch; *D*, spring contacts; *E*, fibre disc; *F*, variable resistance; *G*, resistance lamps; *H*, switch; *J*, fuse.

brass sleeve of which passes through both walls of the chamber and is secured by lock-nuts. A suitable capsule placed in the brass stirrup controls the switch by means of the copper rod pressing against the fibre disc (*E*, Fig. 2) through which the two spring contacts (*D*, Fig. 2) pass. The two contacts are each connected to two of the heating units wired in parallel, the free ends of the strip heaters being all connected to one of the leads from the 100-volt mains. A two-pole, double-throw switch (*H*, Fig. 2) allows either a variable 40 ohm resistance (for the higher temperatures—above 30° C.) or one or more carbon-filament lamps in parallel (for the lower temperatures) to be connected in series with the other lead and the remaining terminal of the switch. By this means the current can be controlled to a suitable degree to ensure that it shall be on and off for approximately the same length of time, this

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condition avoiding too rapid heating or cooling with the resulting wide fluctuations in temperature. A fuse in one main lead is provided for greater safety. The whole circuit is shown diagrammatically in Fig. 2.

The control of humidity presented greater difficulties, but finally the apparatus figured was found to be satisfactory. A glass vessel (*J*, Fig. 1) containing water (or for low humidities dilute sulphuric acid—about 30 per cent.), in which a carbon filament lamp is immersed, is provided with an inlet tube drawn out to a small aperture and reaching nearly to the bottom of the vessel, and an outlet tube bent at right angles and passing through holes in the walls of the cases into the inner chamber. The short piece of tube outside the chamber is constantly heated by a very small flame to prevent condensation. A continuous stream of air is bubbled through this water by means of the apparatus figured, which embodies the principle of the Shenstone apparatus. A glass funnel (*Q*, Fig. 1) (a conical flask with the bottom broken out) is provided with an inlet from the main water-supply and two outlets, one, a straight wide-bore tube leading to the sink, and the other a smaller tube connected to the filter-pump (*R*). By keeping the supply sufficiently rapid for water always to be overflowing down the large outlet a constant head of water is maintained on the filter-pump. The latter delivers a mixture of air and water to the large aspirator (*P*) which is provided with one outlet for air (*O*) and two for water, one a siphon-shaped tube (*S*) of fairly small bore, and the other a wider, straight tube (*T*) provided with a short piece of rubber tubing controlled by a screw-clip. The second of these allows of an approximate adjustment of the outflow, after which the first acts as an automatic regulator in the following manner. There being a constant resistance to the passage of air through the vessel (*J*, Fig. 1), any increase of pressure in the aspirator will result in a faster stream of water through outlet (*S*) and the escape of a little air. For greater safety still the outlet for air (*O*) is carried up to a level higher than the funnel (*Q*) so that in the event of the outflow of water becoming blocked no water can be carried over into the chamber. A long straight tube (*V*) reaching to the bottom of the aspirator serves as a pressure gauge. The apparatus delivers approximately 1 cu. ft. of air per hour to the infection chamber, as measured by the air meter (*W*, Fig. 1).

The hair "hygrostat," which is shown in side view at *E*, Fig. 1, and in plan in Fig. 3, consists of a wooden base about eight inches long and two wide, at one end of which are screwed, one on top of the other, two uprights cut from stout copper or brass sheet. The outer and longer of these carries a screw which presses against the shorter and serves to

adjust the longer for purposes which will be apparent. A bundle of human hairs (*N*, Fig. 3) is connected at one end to this longer upright by passing through a small hole bored in the copper, a knot and short piece of stick preventing it from slipping through, and at the other to a short lever fixed to a horizontal axle (*L*, Fig. 3) pivoted on two more copper uprights screwed to the sides of the wooden base. The other end of the lever carries a long arm (in the actual apparatus the lever and arm were cut out of one piece of sheet tin) bearing at its end a piece

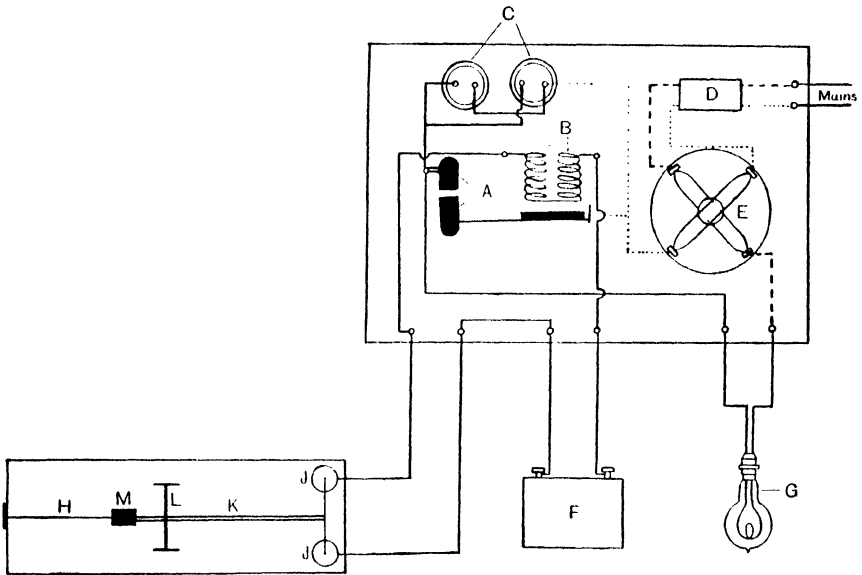


Fig. 3. *A*, carbon contacts; *B*, coils; *C*, lamp-holders (not used in this case); *D*, fuse; *E*, switch; *F*, 4-volt battery; *G*, heating lamp for conditioning vessel; *H*, hairs; *J*, mercury cups; *K*, lever; *L*, axle; *M*, counterpoise.

of platinum wire bent twice at right angles, the ends dipping into mercury cups (*J*, Fig. 3) made by sealing short pieces of glass tubing into holes in a block of wood by means of sealing wax. Pieces of thick copper wire passing through the wooden block make contact with the mercury. The cups are by this means connected with a carbon-contact relay (made by Messrs Gallenkamp and Co.) which switches on the current to the lamp, thus heating the water and causing the air entering the chamber to be charged with more water-vapour. When the humidity within the chamber rises to the required degree, the elongation of the hairs allows the long pointer to rise under the influence of the counterpoise (*M*, Fig. 3) and the relay current is interrupted at the mercury

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cups. The hygrometer can be set for any required humidity by means of the adjusting screw referred to above. A diagram of the relay, hygrometer, and connections to the apparatus is given in Fig. 3.

A constant (though very slow) circulation of the air within the chamber is obtained by connecting the intake of the filter pump (*R*, Fig. 1) to a glass tube passing through the walls near the top of the inner chamber. Dishes containing calcium chloride are placed within the chamber so that the humidity is constantly tending to fall and is constantly raised again by the entry of moist air. This is necessary, since otherwise the air within the chamber would soon become saturated by the transpiration of the plants.

Illumination is provided by a 400 candle-power lamp immersed in a beaker through which water is continuously flowing, the level being maintained by the constant-level overflow (*L*). It was found that this illumination was sufficient to maintain the stomata slightly open.

With this apparatus the temperature within the chamber can be kept constant to within half a degree on either side of the required point, and the humidity within a 4 to 5 per cent. range.

#### *Description of experiments.*

Young cotton plants of strains previously found to be susceptible to the disease were used. At first several varieties were employed, but later 1-2-months-old plants of the Ashmouni variety only were used.

A strong suspension of a young culture of *B. malvacearum* was prepared by shaking up seven loopfuls of the culture with about 20 c.c. of distilled water (a definitely turbid suspension), and the plants thoroughly sprayed with this by means of a nasal atomiser. The strain of the organism used was one isolated in the laboratory from infected seed kindly supplied by Mr R. E. Massey, Mycologist to the Sudan Government. The sprayed plants were then immediately placed in the infection chamber, where they remained for 48 hours, this period having been found to be sufficient for infection to take place. At the end of this period the plants were returned to the glasshouse and examined at intervals. In those cases where infection had occurred, very small water-soaked spots on the leaves were visible after a period varying from two to four weeks. Owing to the fluctuating conditions in the glasshouse during the season it was not thought worth while to record the exact incubation period.

*Results of experiments.*

The date given is that on which the plants were sprayed and placed in the chamber. In every case 48 hrs. was the period allowed in the apparatus.

*Exp. 1.* July 4th. 2 plants, Acala variety. Temp. 35–36° C. Humidity 83–87 per cent. No infection.

*Exp. 2.* July 12th. 1 Ashmouni, 1 Acala plant. Temp. 41–42° C. Humidity 84–87 per cent. No infection.

*Exp. 3.* July 16th. 1 Hartsville, 1 Acala plant. Temp. 30–31° C. Humidity 87–93 per cent. One or two spots on a few leaves.

*Exp. 4.* July 19th. 1 Zagora, 1 American Upland plant. Temp. 24·8–25·8° C. Humidity 84–87 per cent. (but owing to the sticking of the hygrostat reached 95 per cent. for a short time). Heavy infection in two weeks.

*Exp. 5.* July 22nd. 3 Ashmouni plants. Temp. 24–25° C. Humidity 87–92 per cent. Fairly heavy infection in two weeks.

*Exp. 6.* Sept. 24th. 4 Ashmouni plants. Temp. 25° C. Humidity 83–86 per cent. Fairly heavy infection.

*Exp. 7.* Sept. 30th. 3 Ashmouni plants. Temp. 30° C. Humidity 83–86 per cent. Slight infection (2 leaves of one plant) after three weeks.

*Exp. 8.* Oct. 15th. 3 Ashmouni plants. Temp. 32° C. Humidity 83–86 per cent. Very slight infection on two plants after three weeks.

*Exp. 9.* Oct. 19th. 3 Ashmouni plants. Temp. 25° C. Humidity 69–71 per cent. Slight infection on two plants in three weeks.

*Exp. 10.* Oct. 25th. 3 Ashmouni plants. Temp. 27–28° C. Humidity 65 per cent. (but twice up to 70–74 per cent. for short time). Slight infection in three weeks.

*Exp. 11.* Nov. 2nd. 3 Ashmouni plants. Temp. 28° C. Humidity 64–66 per cent. No infection.

*Exp. 12.* Nov. 16th. 6 Ashmouni plants. Temp. 28–29° C. Humidity 58–61 per cent. No infection.

Since in each case at least two plants were used and each plant bore at least six leaves the surfaces of which were sprayed with an atomised suspension, it is clear that considerable replication was obtained, even though each separate experiment was not repeated. Ample opportunity for infection was provided since both sides of every leaf were thoroughly wetted with the suspension.

It is seen that both temperatures and humidity can apparently act as limiting factors.

Table I.

Exp. no.	Average temperature ° C.	Average relative humidity %	Infection
2	41	85	—
1	35	85	—
8	32	81	+
3	30.5	89	+ +
6	30	85	+ +
4	25	85	+ + + +
7	25	85	+ +
5	24.5	90	+ + +
9	25	70	+ +
10	27.5	65*	+
11	28	65	—
12	28.5	60	—

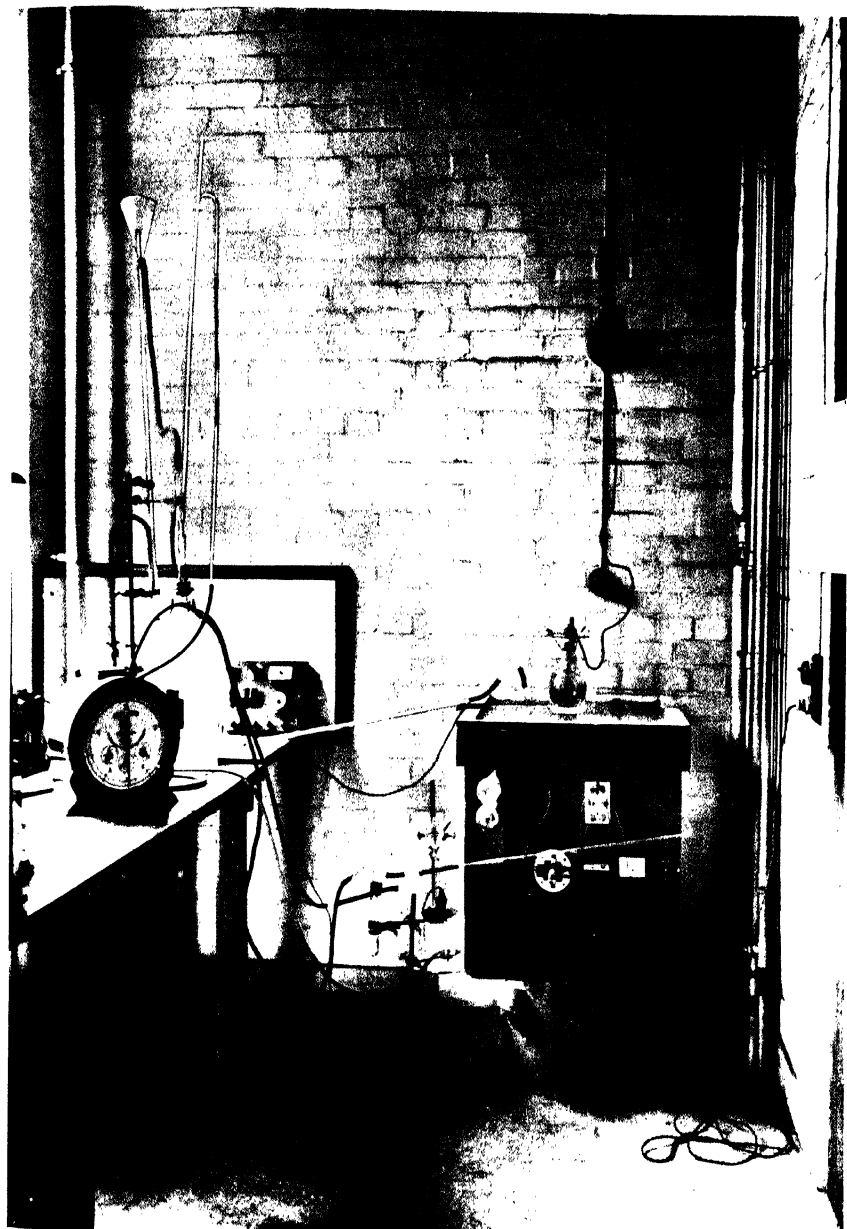
\* Exps in which humidity reached 70–74 per cent. for short time.

— = no infection; + = very slight infection; + + = slight infection; + + + = moderate infection; + + + + = heavy infection.

The results are shown in Table I, in which the experiments are arranged in two groups, the first with a high humidity (over 80 per cent.) and the second including those where the humidity was under 70 per cent. In the first group it is seen that above 32° C. no infection was obtained, while at that temperature very slight attack only was possible. Under such conditions of high relative humidity it appears that this temperature is the maximum at which infection can occur.

In the second group it is found that while slight infection was possible at 70 per cent. relative humidity, none was obtained at 65 per cent. humidity at a temperature of 28° C. (at which temperature infection occurs freely at high humidities).

Further experiments are being carried out to determine whether the limiting temperature varies with the degree of humidity and *vice versa*. It is possible, for example, that at the lower humidities infection may occur at lower temperatures such as 23–25° C. In the meantime it is clear that both factors are of considerable importance in the development of the disease. Temperature, of course, can hardly be controlled under field conditions, but cultural methods, such as good spacing of the plants, avoidance of overcrowding, even possibly the thinning of branches, which will help to prevent humid atmospheric conditions around the plant, may be of considerable importance in the control of the disease.



UGHTON.---ON THE DEVELOPMENT OF THE ANGULAR LEAF-SPOT DISEASE OF COTTON (pp. 333-341).



## SUMMARY.

1. The serious disease of cotton caused by *Bacterium malvacearum* E. F. S. is associated with unfavourable climatic conditions.
2. A description of an apparatus for controlling air temperature and humidity within a chamber is given.
3. At humidities above 80 per cent. relative saturation the limiting temperature for attack by *B. malvacearum* was 32° C., above which infection did not occur.
4. At 70 per cent. relative humidity infection was slight at 25° C.
5. At lower humidities no infection was obtained at a temperature of 28° C.

## REFERENCES.

- (1) ATKINSON, G. F. (1891). Black Rust of Cotton. *Alabama Agr. Exp. Sta. Bull.* 27, 16 pp. 2 pls.
- (2) SMITH, ERWIN F. (1901). The cultural characters of *Pseudomonas hyacinthi*, *Ps. campestris*, *Ps. phaseoli*, and *Ps. Stewartii*—four one-flagellate yellow bacteria parasitic on plants. *U.S. Dept. Agr. Div. Veg. Phys. and Path. Bull.* 28, p. 153.
- (3) — (1920). *The Bacterial Diseases of Plants*. Saunders, Co., Philadelphia, pp. 314–339.
- (4) ATKINSON, G. F. (1892). Some diseases of cotton. *Alabama Agr. Exp. Sta. Bull.* 41, pp. 1–65.
- (5) ROLFS, F. M. (1915). Angular leaf-spot of cotton. *S. Carolina Agr. Exp. Sta. Bull.* 184, pp. 1–30.
- (6) SNOWDEN, J. D. (1926). The occurrence of angular leaf-spot of cotton in Uganda. *Dept. Agr. Uganda Circ.* 17, pp. 1–12.
- (7) NAKATA, K., NAKAJIMA, T. and TAKIMOTO (1924). Studies on angular leaf-spot of cotton in Korea. *Bull. Agr. Exp. Sta. Chosen*, 10. Abs. in *Japanese Journ. Bot.* 11, 4, 1925.

## EXPLANATION OF PLATE XVIII.

Photograph of apparatus.

(Received February 14th, 1928.)



576 . 851 . 4 B . *malvacearum* . 094 + 633 . 51—2 . 3

*The Morphology and Cytology of Bacterium malvacearum*, E.F.S.

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(Communicated by Sir John Russell, F.R.S.—Received September 6, 1929.)

[PLATES 24–26.]

*Introductory.*

During the course of studies on the angular leaf-spot disease of cotton, caused by the organism *Bacterium (Pseudomonas) malvacearum*, E. F. Smith, the production was constantly observed of bacterial forms differing from the normal structureless cell as seen in stained films from 24-hour old cultures of the organism. Of these the most conspicuous feature was the presence of deeply staining structures within the body, especially in preparations from cultures more than 4 days old. For some time these unusual forms were considered as artefacts produced by the staining technique, or else as contaminations. Observations of living bacteria from cultures derived from single cells, and especially evidence obtained from the examination of unfixed wet films by a technique described below, proved conclusively that neither of these explanations was correct, and that the organism possessed hitherto unrecorded internal structure and variations in morphology.

An enormous mass of literature on the subject of bacterial structure and variation has now accumulated. Reference need only be made to the review by Löhnis (8) of the literature on the subject up to 1918, to the analysis of the problem of microbial dissociation by Hadley (5), to the relevant papers in the symposium edited by Jordan and Falk (6), and to the work of Enderlein, most of which has been brought together in his book "Bakterien-Cyclogenie." (3).

The purpose of this paper is to present evidence for adding another bacterial species to those for which variation from the so-called "normal" type of reproduction has been shown to occur. It is of interest that *Bacterium malvacearum* belongs to the rather small group of plant pathogens, since with the exception of the investigations by Levine (7) and Rosen (10) on *B. tumefaciens* little work has been done on this group.

So far as can be ascertained from the literature, *Bact. malvacearum*, first described in 1901 under the name of *Pseudomonas malvacearum* by Erwin F. Smith (11), has always been reported as an invariably structureless slender rod multiplying solely by transverse fission. Faulwetter (4) states specifically that "no capsules, granules, or endospores have been demonstrated." That the phenomena to be described are not peculiar to the particular strain of the organism used has been proved by examination of a number of other strains obtained from sources as widely apart as America, China and the Sudan. Although there is some evidence that the strains differ in detail, the general principles of development have been found to be the same.

#### *Material and Methods.*

In these studies a single strain of *Bact. malvacearum* has been used, originally isolated by the writer from infected seed supplied by the courtesy of Mr. R. E. Massey, Botanist to the Sudan Government. The purity of the strain has been ensured, not only by repeated platings, but in addition the culture has been twice restarted from a single cell isolated by means of the Dickinson "Micro-Isolator" (1). The strain is a strongly virulent one and has been used for infection experiments concurrently with these investigations.

In order to obtain uniformity of results and to eliminate the possibility of changes produced by varying external conditions, the greater part of this work was carried out on agar slope cultures on standard media incubated at a constant temperature of 25° C., which is near the optimum for the organism. The media used were a standard potato-extract containing 1 per cent. of saccharose, and a synthetic medium. The potato-extract is prepared by boiling 200 gms. of sliced potato to a pulp and straining through muslin. The extract is made

up to 1 litre, 1·5 per cent. of agar added, the whole autoclaved at 15 lbs. pressure, filtered through paper-pulp under a vacuum, 1 per cent. of saccharose added and the medium tubed and sterilised at 115° C. for 15 minutes. Some hydrolysis of the sugar doubtless occurs, but as it has been found that the substitution of dextrose or levulose for saccharose has no effect on the organism the latter sugar is used on account of the ease with which it may be obtained in a pure condition. The synthetic medium was devised originally for the purpose of studying nutritional phenomena. The composition is as follows :—

	Per cent.
Di-potassium phosphate ( $K_2HPO_4$ ) .....	0·1
Potassium nitrate ( $KNO_3$ ) .....	0·2
Magnesium sulphate ( $MgSO_4$ ) .....	0·1
Sodium chloride ( $NaCl$ ) .....	0·1
Dextrose .....	1·0
Agar .....	1·5

The phosphate, chloride and nitrate are dissolved together in a little water, the magnesium sulphate dissolved separately, the two solutions mixed and made up to the correct volume with water. The agar is then added, melted in the autoclave at 115° C., and the medium filtered through paper pulp under pressure. The sugar is then added and the medium brought to a reaction of 7·0  $P_H$ . It is then tubed and sterilised at 115° C. for 15 minutes.

The organism has been found to grow freely on this medium and to produce the same forms as on the potato-extract. The medium has the advantage of being constant in composition, reasonably well buffered, and permitting of alterations in the nutritional factors without difficulty. Except where otherwise stated these two media have been used throughout the investigation. It is, of course, well known that with bacteria as with fungi certain stages of development are produced only in response to altered conditions of the medium or of the external physical factors, but it was thought better in this study of the primary morphology of *Bacterium malvacearum* to keep all factors constant and to leave the investigation of the effect of altered conditions to a later date.

In the earlier part of the enquiry the work was carried out on dried films prepared in the usual way, fixed and stained by a variety of methods. When it became apparent, however, that the visible appearances bore some real relation to an internal structure in the organism, it was recognised that some technique must be employed which would avoid the drastic processes involved

in the preparation of a dried film. It is obvious that in the process of drying alone, even if the bacteria have been previously fixed by a chemical reagent, some degree of distortion is produced. Further, in the staining of a dried film there is always the risk of deposition of precipitates, staining of particles adhering to the slide, distortion due to the concentration of the stain, and so on. In fact, with dried films, even with the most careful technique it is never possible to be certain that appearances seen represent real structures present in the living organism and not artefacts produced by the method of preparation. Some control of the observations, it is true, can be obtained from examination of the living cells, but their extremely small size precludes the possibility of exact observations in this manner. This point will be dealt with later.

Dobell (2) in his studies of nuclear behaviour in bacteria found no essential difference in appearance between bacteria stained in wet films and the same organisms in stained dry preparations. With *Bact. malvacearum* this is not the case; even after preliminary fixing dried films invariably show some degree of shrinkage of the internal structure, associated with a flattening and consequent broadening of the cell-body. The photomicrographs on Plate 24 (Nos. 5 and 6) illustrate this point. It is true that in the dried films the central structures to be described are much more sharply defined, but the fact that shrinkage has obviously occurred makes it impossible to interpret the appearance as representing the true forms of the structures, although the clarification of outline is of value in confirming the observations on the stained wet films.

The method finally adopted was a modification of that used by Nakanishi (9) in his studies on nuclear structures in bacteria. Chemically cleaned slides which have been kept in ammonia-alcohol, are further cleansed by flaming. A drop of the stain to be used (Ziehl's carbol-fuchsin diluted with an equal quantity of water has been most employed) is placed at one end of the slide, and a thin film of the stain made by drawing the edge of a strip of typewriting paper over the drop and along the slide. This film should dry rapidly and evenly and be barely perceptible when held to the light. A small drop of sterile water is then placed in the middle of a flamed thin cover-glass, touched with a wire carrying the organisms from the culture, and the cover-slip inverted and dropped on the stained slide. The mount is then sealed with vaseline by means of a camel-hair brush, or, if more permanence is required, with gold size.

The organisms take up the stain quite slowly if the film is correctly prepared, and the process of gradual intensification can be watched under the microscope.

The structures appear successively in the order of their affinity for the stain, and this makes their visual differentiation less difficult. This phenomenon of progressive staining, coupled with the fact that the organisms are not fixed by drying or subjected to any other distortional process, makes it reasonable to assume that the appearances seen represent real structures and not artefacts. This is further confirmed by the fact that the appearances are practically the same with all the usual basic aniline dyes. Preparations have also been made by the same method with bacteria previously fixed by corrosive sublimate or picric acid; no difference in the appearances presented can be observed.

*Structural Changes in Young Cultures of Bact. malvacearum.*

*General Appearances.*—Examination of a culture 24 hours old on either of the standard media, stained by the method described, shows a preponderance of slender rods, which stain more or less evenly and deeply. These conform in every respect to the typical descriptions of the organism given by previous workers. In size they vary from about 1.5 to 4.0  $\mu$  by 0.5 to 0.9  $\mu$ , and in shape they are cylindrical with rounded ends. If the stain is not too intense, however, or if the preparation is examined immediately it is made, before the staining process is complete, a certain number of the cells are seen to contain a deeply staining spherical granule (rarely two or more) usually situated towards one end of the cell and conspicuous by reason of its greater affinity for the stain. This structure is soon obscured by the staining of the rest of the body.

The same general appearance is presented in films from cultures up to about 3 days old, with the exception that the cells containing granules become more numerous and the latter more conspicuous, by reason of the tendency of the cell-body to stain less deeply. After the lapse of 3 to 4 days the cells in the culture lose their power to stain evenly and another structure becomes visible. This is centrally placed and presents a different appearance in different cells as described on p. 474. The change in staining reaction begins with the bacteria at the top of a slope culture and progresses downward, so that in a culture about 72 hours old films prepared from the top of the slope will show the differentiated staining, while those from the water of condensation will still resemble preparations from 24-hour cultures. In a time varying from 5 to 8 days the whole culture shows the differentiated reaction. The significance of this point will be discussed in the consideration of the nature of the structures.

Concurrently with the appearance of the central body the granules previously

referred to become very conspicuous, by reason of their intense stain in contrast with the light staining of the ends of the cells. These granules and the changes they undergo are described on p. 479.

*The Central Structure.*—This body becomes most clearly and sharply defined in cultures about a week to 10 days old, while the cells are of the normal size and shape and before the changes which occur in old cultures have begun to appear. It is readily observable that the central structure bears some relation to the condition of the cell with regard to the division process. In immature cells that have clearly been recently formed by the division of a mother cell the structure appears usually as a homogeneous, more or less spherical, centrally placed body, staining with basic dyes, but less intensely than the granules referred to previously (Plate 24, figs. 1, 3 and 4). In some cells apparently in the same stage, however, the body presents the appearance of a four-lobed structure, or alternatively of four very small bodies all close together in a "tetrad" formation.

It is necessary here to emphasise the optical difficulties encountered in the examination of these internal structures. The extreme limit of resolution for a well-corrected lens-system is given approximately by the quotient of half the wave-length of the light used divided by the effective numerical aperture of the lens. That is, the expression

$$\frac{\lambda}{2 \times \text{N.A.}}$$

gives the least distance between particles that can be observed by the given lens working under the best conditions. It follows that if two particles of any size are separated by a distance less than this, it will be impossible to resolve them by that lens and they will appear as a single particle.

Working with an apochromatic objective of N.A. 1.40 in monochromatic green light ( $\lambda =$  approximately 5000 Ångstrom units  $= 0.5 \mu$ ) the limit of resolution would, theoretically, be about 0.17 to 0.18. In practice this degree of resolution is rarely attained, since it is not usually possible to utilise the full theoretical numerical aperture of the lens, and for most purposes the limit may be put at about  $0.2 \mu$  to  $0.25 \mu$ .

Now the diameter of a cell of *Bact. malvacearum* is usually about  $0.6 \mu$ , so that the central structure will be somewhat less than this, say  $0.5 \mu$ , and the diameter of the separate components of the "tetrad" (if a real appearance) must be of the order of  $0.2 \mu$ , or perhaps a little more, while their distance apart will be less than this. It will be seen therefore that the resolution of

this structure under usual conditions presents an almost insuperable problem, and it must be admitted that, although it is *seen* as a "tetrad," its existence as a *real* "tetrad" formation is conjectural.

In cells which are obviously in the process of division the central structure presents a different picture. Normally in cells of this stage it appears as an elongated "dumb-bell," comprising two rounded bodies, one in each half-cell with a connecting strand between (Plate 24, figs. 1 and 2). Again in some cells in this condition each end of the "dumb-bell" appears more or less bi-lobed or double.

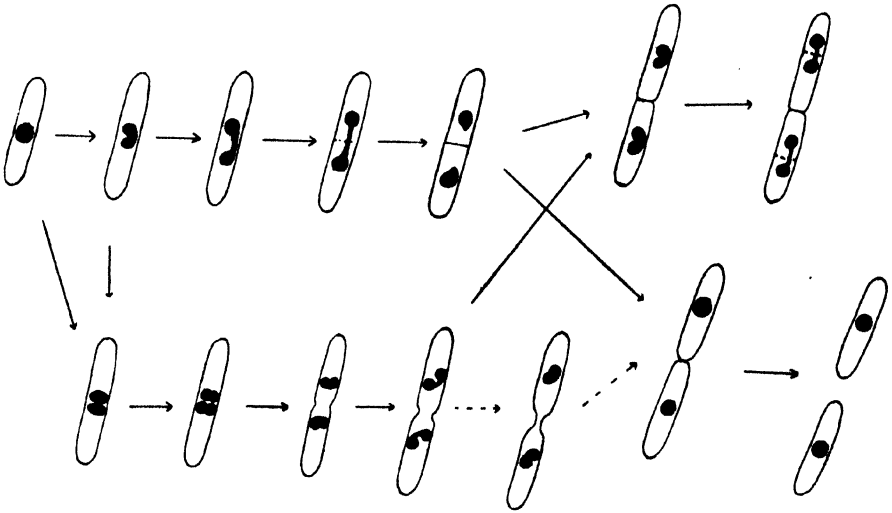
These two (or possibly four) conditions of the cell give fixed points of reference in an attempt to construct a consecutive story from the other appearances. In some cells the structure appears elongated and with only slight indications of a "dumb-bell" shape; this being commonly associated with an elongation of the whole cell such as occurs before the usual transverse fission takes place. Again in other cells the central structure appears to have divided completely into two before the elongation of the cell has progressed far.

These last two appearances are apparently associated with two distinct methods of fission of the cell. In the first case where the central structure elongates with the cell the latter seems to divide by a pinching through, much as if it has stretched and given way in the middle. In the second case, which seems to be derived from the previously described "dumb-bell" by separation of the two halves, the division of the cell appears to be accomplished by the laying down of a transverse wall across the cell between the two halves of the central body.

Reference to the photomicrographs (Plate 24) makes clear the various changes through which this central structure may pass. It seems reasonable to conclude that these different appearances represent phases in a division-cycle (possibly entailing two alternative modes of division) of the originally single central structure, and that this cycle (or cycles) is intimately correlated with the normal fission-processes of the cell. In some cases the central structure apparently divides more or less completely before the somatic division has progressed far, but in every case, so far as has been ascertained, the two processes of division are either simultaneous or immediately consecutive.

From these observations alone it seems justifiable to construct a coherent story for the division process, with a possible alternative. An attempt has been made on these lines to connect the various appearances into two such division-cycles; these are represented diagrammatically in text-fig. 1.

Before passing to a consideration of the probable nature of these structures



TEXT-FIG. 1.—Diagrammatic representation of hypothetical alternative division-cycles in vegetative cell of *Bacterium malvacearum*.

reference should be made to the phenomena observed in examinations of the living dividing cells.

#### *Observations on Living Cells.*

The examination of living bacteria for evidence of their internal structures and division-processes is attended by many very great practical difficulties. The difficulties of optical resolution already referred to apply with even greater force in this case, since in the examination of the living unstained organism it is usually impossible to utilise more than at the most some two-thirds of the total aperture of the objective. This is in part due to the fact that the refractive index of the bacterial body and of its internal structures, being not very different from the surrounding medium, makes it necessary to restrict the aperture of the illuminating cone from the sub-stage condenser. In addition to this purely optical difficulty the problem is complicated by the necessity for the avoidance of contamination, combined with the provision of sufficient oxygen for growth, and at the same time the prevention of desiccation of the mount during the period of examination.

Probably the ideal would be an examination of the dividing bacteria under dark-ground illumination, for under these conditions it is theoretically possible with some types of reflecting condenser to utilise the full aperture of an oil-immersion lens, resulting in a great improvement in resolution. At the same time this method of visual examination renders it possible to make out the

structures much more clearly than by transmitted light. For high-power dark-field illumination, however, it is necessary to have a continuous system of approximately the same (or at least a high) refractive index between the front lens of the condenser and the objective. This condition is sufficiently nearly satisfied if the organisms are in a continuous film of liquid between the slide and the cover-glass. Despite numerous attempts, however, it has not yet been found possible to obtain a continuance of growth under this condition. Division has only been seen to occur where the bacteria had access to free oxygen, as when lying on the surface of a film of agar medium.

Recourse has therefore been made to transmitted light, and although it has not yet been possible to define the structures exactly under these conditions, sufficient evidence has been obtained to confirm the correlation between the division of the central structure and cell-fission. The method found most suitable for these observations was as follows:—An ordinary thin glass slide is perforated with a hole half-an-inch in diameter and on the underside is cemented a thin cover-slip, so as to make a shallow chamber. A drop of filtered sterile melted medium is allowed to run down an extra thin cover-glass, and when this has set the excess of the agar film is cut away with a sterile scalpel, leaving a small square of very thin film in the middle of the slip. This is touched with a wire carrying the organisms and inverted over the shallow chamber. The mount is then sealed with vaseline and examination made for an area where only a few organisms are present in the field. Most modern 1/12-inch objectives will work through this thickness, provided that only extra thin (No. 0) cover-slips are used.

Using this method it was possible to make out the central body as an ill-defined refractive structure. Continuous observation of cells during the process of division shows that this structure at first elongates, then becomes dumb-bell shaped, and finally divides into two, each half passing into one of the daughter-cells. For the reasons given, resolution of the central structure into its details has been impossible, but the evidence is conclusive for the presence and division of the central structure coincidently with the division of the cell.

#### *Significance of the Central Structure.*

The evidence of correlation between the division of the central body and that of the cell immediately raises the analogy of nuclear division. The affinity for basic dyes, the position in the cell, and the shape and behaviour of the central structure, all lend support to the hypothesis that these central structures are of the nature of nuclei which undergo a process of division.

In this connection the work of Enderlein (3) referred to in the introductory part of this paper is of much interest. He concludes that the nuclear unit ("Mych") is the caryological constituent of the primitive cell ("Mychit"). The nucleus is spherical or oval in form and occupies a position close to the wall of the cell. In the coccus there is but one nuclear body, while in all other forms there are two or more. The diameter is  $0.1\ \mu$  to  $0.25\ \mu$ . It contains no chromatin and stains hardly any more strongly than the cytoplasm of the cell. The nuclear body is observable only when the cell holds but little reserve food-substance. The latter is distributed throughout the young cell in ultra-microscopic granules, the "*trophoconia*," and constitutes the "chromatic" material of the cell, staining strongly because of its high content of nucleic acid and nucleo-proteins. When this food reserve substance is abundant, as in young cultures, the material forms a dense aggregation about the nuclear body, forming a large strongly staining spherical body, the "*trophosome*." Even when nearly all the reserve has been used up the last remnant clings tenaciously around the nucleus, forming the element known as the "*trophosomelle*." Large amounts of food reserve substance, which may conceal not only the nucleus but also the trophosome or the trophosomelle, may be removed by alcohol. Under these conditions, when properly stained, it is observed that, in coccus forms, only a single point takes the stain. In other forms two or several such bodies become evident. These are often at the poles of the cell and represent the true bacterial nucleus or nuclei. After cell-division the heavily staining reserve substance soon appears in the daughter-cells.

It will be seen how closely the phenomena described for *Bact. malvacearum* fit this general hypothesis. Using the technique described by Enderlein it has been found that the central structures gain in clearness by treatment with alcohol, and the "tetrad" appearance referred to becomes even more evident. Whether it will ultimately be possible to resolve the apparently single body into two in all cases remains to be seen. In that case the two alternative cycles of changes suggested in text-fig. 1 would be resolved into one only. At present it appears as though both were possible.

On all points one is led to the conclusion that these central structures represent either true nuclei or possibly (following Enderlein's theory) nuclei imbedded in a mass of "chromatic" food reserve substance. Further these "nuclei" undergo a division process, either immediately prior to, or coincidently with, the division of the cell-body, the latter division being related to the nuclear process.

*Gonidia.*

Reference has already been made to other bodies, distinct from the central ("nuclear") structures which have been described, found commonly in the cells of young cultures of *Bact. malvacearum*. These are spherical granules, up to  $0.3\ \mu$  or so in diameter, characterised by their intense affinity for basic dyes. In a wet film prepared by the technique described these granules are the first to take up the stain and are usually intensely stained before the other cell-structures are appreciably coloured. This fact allows of their differentiation from the "nuclear" structures, with which they might otherwise be confused when produced near the middle of the cell. They may be formed in any position, but most commonly occur rather towards one end of the cell, occasionally even occupying a polar position. Usually they are found singly, but two or more may occur in one cell.

The granules appear to be formed either closely adpressed to, or actually in, the cell-wall, and as they grow in size protrude slightly when seen in profile (Plate 25, fig. 1). Liberation appears to be accomplished in one of two ways, either by simple extrusion through the wall, or else by growth on an elongated stalk, which may reach a length of 1 or  $2\ \mu$  (Plate 25, figs. 3 and 4). It has not been possible to ascertain whether this stalk is formed by an evagination of the cell-wall or by actual growth of a new organ *sui generis*. The former hypothesis is lent support by the fact that the granule appears to be invested by a membrane or sac which is continuous with the stalk. This sac is not clearly visible surrounding the granule, but after liberation of the latter the stalk, which remains attached to the parent cell, commonly carries at its end the collapsed remains of the membrane, appearing as a faintly staining irregular fragment.

The photomicrographs (Plate 25) show these various stages of the stalked granules and old granules bearing fragmented membranes. The liberated granules can be seen free in the culture under dark-ground illumination. Owing to their extremely small size their Brownian movement is very great and it has not been possible to prove conclusively whether or not they are truly motile. Flagellæ have not been demonstrated attached to the granules.

Bodies of this nature have been observed by other workers in all types of bacteria, including an immense number of species. An exhaustive review of observations and conclusions of a great number of workers, and a critical analysis of the data, are given by Löhnis in his "Studies upon the Life-cycles of the Bacteria" (8). He concludes that the Babes-Ernst "metachromatic

granules," the "microsomes" of Klebs, the "Eier" of Ehrenberg, the "Blastia" of Perty and the "infective granules," "swarm-spores," "conidia," and many of the "buds" and "umbels" of other writers, are all of the same type, which he classes as "gonidia." This term was originally applied to reproductive organs of the lower algæ, and "gonidia" were defined by Sachs as organs of asexual reproduction, formed by contraction of the plasmatic cell content, which leave the parent cell either by breaking the cell-wall or become liberated when the cell dissolves.

That the granules observed in *Bact. malvacearum* are of this type there can be little doubt, and it seems reasonable to regard them as true gonidia, despite the fact that they have not as yet been observed to grow out into normal rods. This point, together with the developmental history of the organism within the plant, will be dealt with in a later paper. It may, however, be pointed out here that both the "nuclear" structures and the gonidia have been demonstrated in the bacteria occurring within plant lesions.

#### *Changes Observed in Old Cultures.*

The general appearances described above persist in cultures for some weeks, with the exception, however, that a greater variability in size and shape of the individual organisms becomes more and more apparent. There is a general tendency towards the production of smaller forms, averaging about  $2\ \mu$  in length, but a certain number of larger and more oval forms are also produced. After the lapse of about 6 weeks the variability becomes very marked. While the majority of the cells are either of the normal type or smaller, a small percentage (5–15 per cent.) of "giant cells" are found. These are of various sizes and shapes, up to as much as  $5\ \mu \times 10\ \mu$  (Plate 26, figs. 1 and 2). Many of them show no internal structure and stain only lightly, while others may contain numerous staining granules; they resemble the so-called "involution" or "degeneration forms" so commonly described for many species of bacteria and appear to be produced in response to the unfavourable conditions for growth. This is borne out by an experiment on the growth of the organism on the synthetic medium described earlier in this paper, using a variety of sugars in place of dextrose. The most striking effect was produced by the use of maltose. On this medium growth is very scanty and soon ceases. Examination of the culture microscopically shows the presence of large numbers of giant "balloon-like" cells of a great variety of shapes and internal structures. The significance of these "giant-cells" has not yet been worked out, but it

seems unlikely that they represent any real stage in the normal life-cycle of the organism. They have not as yet been observed in the plant lesions.

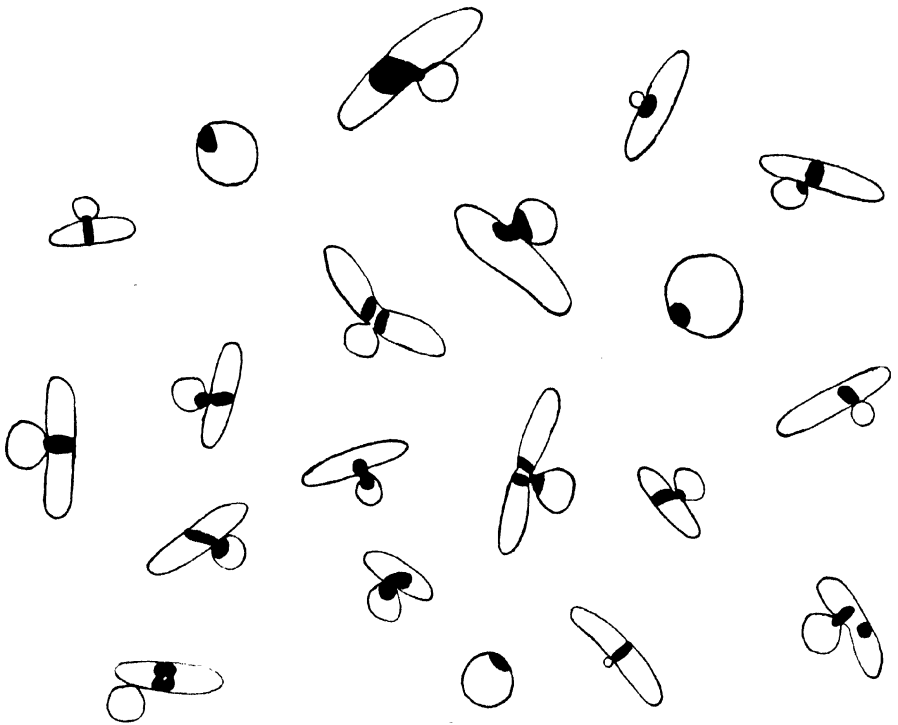
A common appearance in these old cultures is the occurrence of rather large, swollen, oval cells, occurring in pairs attached at their ends, and remarkable for the fact that they appear usually at a constant angle to one another. Whether they represent daughter-cells of an incomplete but normal division, or are produced by some other process, is not yet clear. Several of these "angled" forms may be seen in Plate 26, fig. 1.

Associated with these diverse types produced in old cultures is yet another form. These are spherical or slightly pear-shaped cells 2-3  $\mu$  in diameter and containing a single deeply staining point. In cultures which have been sealed with paraffin wax immediately after inoculation and left for several months these coccus-like forms are produced in great numbers. In such a culture the cells are all of two forms, very short rods of the typical structure and these spherical cells. The mode of production of the "cocci" has been followed in stained films from these old cultures prepared by the technique described.

#### *Method of Formation of the Cocci.*

The spherical coccus-like forms are produced by a process of budding of the normal or somewhat swollen rods and oval forms in old cultures. The bud appears first as a small protuberance from the wall of the parent-cell, which enlarges and swells into a spherical form. The bud is usually formed at the point in the cell where the "nucleus" lies. During its growth it remains attached to the parent cell by a very short and narrow neck, which provides continuity between the plasma of the cell and that of the bud. As the bud increases in size a portion of the "nucleus" of the parent-cell passes through the narrow neck and ultimately becomes abstricted as the "nucleus" of the free coccus. In this respect the "nucleation" of the coccus appears to differ from the process observed in ordinary vegetative division. In the latter a symmetrical division of the "nucleus" takes place about the transverse axis of the cell, while in the former a portion of the parent "nucleus" seems to squeeze through the narrow channel of attachment and then to become cut off by the separation of the bud. This passage of a part of the "nucleus" into the bud is shown in Plate 26, figs. 3 and 4, while text-fig. 2 shows drawings of cocci in various stages of development.

The biological significance of these coccus-like reproductive forms is not yet clear. The fact that they are normally produced only under adverse conditions for vegetative development of the culture suggests that they may represent



TEXT-FIG. 2.—Drawings of "cocci" in various stages of formation. Magnification about 17,000 diams.

some more resistant form of the organism than the normal vegetative rod. If this is so it would help to explain the many inconsistencies observed by different workers on the resistance of the organism to various lethal agents, such as temperature, desiccation, and disinfectants, and they (or the gonidia) might prove to be a factor in the persistence and spread of the disease in the field. The fact that no "normal" resistant spores are formed by organisms of this group has always been a stumbling-block in attempted explanations of the method of persistence of the various diseases caused by them from season to season.

#### Summary.

1. *Bacterium (Pseudomonas) malvacearum*, E.F.S., has hitherto been described as possessing no internal structure or reproductive bodies, and as multiplying solely by transverse fission.

2. A technique is described for staining the bacteria without previous drying or fixing.

3. Using this technique several structures and a variety of different morphological forms have been observed in *Bacterium malvacearum*.

4. An internal central structure is described, which passes through a division cycle, which is correlated with the division of the cell-body, and is suggestive of nuclear division.

5. Small granules with a strong affinity for basic dyes are described. These are formed in the wall of the cell and are liberated by simple extrusion, or grow out on a stalk from the end of which they are freed. These bodies resemble the "gonidia" of other writers.

6. The occurrence and mode of formation of spherical coccus-like bodies in old cultures is described.

7. "Giant-cells" and other atypical forms have been found to occur in old cultures.

#### REFERENCES.

- (1) Dickinson, S., "A simple method of isolating and handling individual spores and bacteria." 'Ann. Bot.,' vol. 40, 157, pp. 273-274 (1926).
- (2) Dobell, C. C., "Contributions to the cytology of the bacteria." 'Quart. Jour. Micr. Sci.,' vol. 56, pp. 395-506 (1910-11).
- (3) Enderlein, G., "Bakterien Cyclogenie," Berlin (1925).
- (4) Faulwetter, R. C., "The angular leaf-spot of cotton." 'S. Carolina Agr. Expt. Sta.,' Bull. 198 (1919).
- (5) Hadley, P. J., "Microbic dissociation." 'Jour. Inf. Dis.,' vol. 40 (1927).
- (6) Jordan, E. O., and Falk, I. S., "The Newer Knowledge of Bacteriology and Immunology." Chicago (1928).
- (7) Levine, M., "Morphological changes in *Bacterium tumefaciens*." 'Science,' vol. 62, p. 424 (1925).
- (8) Löhnis, F., "Studies upon the life-cycles of the bacteria. Part I.—Review of literature 1838-1918." 'Mem. Natl. Acad. Sci.,' vol. 16 (1921).
- (9) Nakanishi, K., "Vorläufige Mitteilung über eine neue Färbemethode." 'München. med. Wehnschr.,' vol. 47, p. 187-188 (1900).
- (10) Rosen, H. R., "Morphological notes, together with some ultra-filtration experiments on the crown-gall pathogene, *Bacterium tumefaciens*." 'Mycologia,' vol. 18, pp. 193-205 (1926).
- (11) Smith, Erwin F., "The cultural characters of *Pseudomonas hyacinthi*, *Ps. campestris*, *Ps. phaseoli*, and *Ps. stewarti*—four one-flagellate bacteria parasitic on plants." 'U.S. Dept. Agr. Div. Veg. Phys. and Path.,' Bull. 28 (1901).

#### EXPLANATION OF PLATES.

##### PLATE 24.

FIGS. 1-4.—2 to 4-week culture of *Bacterium malvacearum*, showing stages in the division of the central structure. Preparations made by the wet-film method. Stained carbol-fuchsin.  $\times 2000$  approx.

FIGS. 5 and 6.—Dried films from 2-week cultures, showing shrinkage of internal structures and distortion of cells. Stained Bleu de Roux. Fig. 5,  $\times 1500$ ; fig. 6,  $\times 2200$  approx.

PLATE 25.

FIGS. 1 and 2.—2-week culture, showing gonidia in the cells and in process of liberation.

Fig. 1,  $\times 2150$ ; fig. 2,  $\times 1500$ . Wet-film preparation.

FIGS. 3, 4 and 5.—Ditto. Stalked gonidia and remains of stalk and membrane.  $\times 2200$ , approx. Wet-film preparation.

FIG. 6.—Living cells under dark-ground illumination, the lowermost cell showing a gonidium in the wall.

PLATE 26.

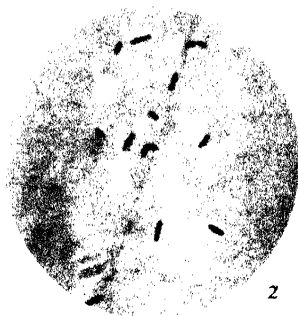
FIGS. 1 and 2.—4-week cultures, showing production of "giant-cells," "angled" pairs and atypical forms. Wet-film preparation.

FIGS. 3-6.—4 to 8-week cultures, showing production and method of "nucleation" of "cocci." Fig. 4,  $\times 3000$ ; others,  $\times 2000$ , approx. Wet-film preparation.

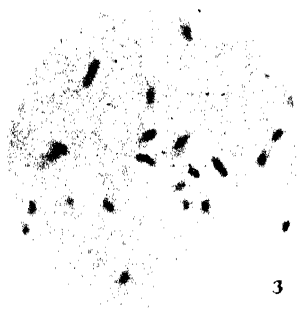




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2



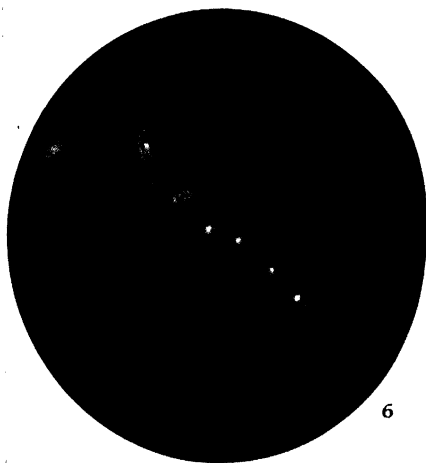
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4



5



6



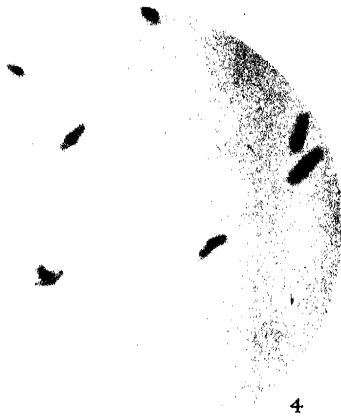
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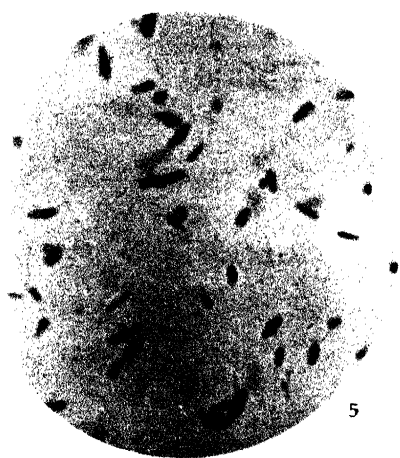
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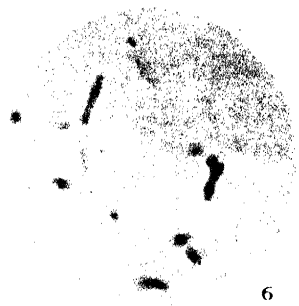
3



4



5



6



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## THIONIN AND ORANGE G FOR THE DIFFERENTIAL STAINING OF BACTERIA AND FUNGI IN PLANT TISSUES

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(With Plate VII.)

IN the course of studies on the disease of cotton caused by the organism *Bacterium malvacearum* the need arose for a method of tracing the progress of the organisms through the tissues of the host. Sections were stained by many different methods, most of the well-known combinations, such as the Planeze stain as used by Vaughan<sup>(1)</sup>, the Giemsa stain as modified by Wright and Skoric<sup>(2)</sup>, Ziehl's carbol-fuchsin and light green, iron alum haematoxylin and so on, being tried, as well as a number of other combinations. None, however, gave a really satisfactory result.

The organism produces a considerable amount of slime, and this stains very readily, with the result that the bacteria are obscured by the diffuse stain. Further, none of the combinations referred to differentiated between the slime and the host tissues.

Thionin is well known as a stain for differentiating mucin in animal tissues owing to its high metachromasy, mucin being stained pink and other tissues shades of blue and purple. Used in aqueous or phenol solution on diseased plant tissue it gave very promising results, but the required degree of differentiation of host and parasite was not obtained owing to the intense staining of the host tissue. Orange G in alcoholic solution was, however, found to be a good differentiating agent and at the same time acted as an excellent counter-stain for the cellulose walls. The technique adopted was as follows:

### PARAFFIN SECTIONS.

- (1) Xylol to remove wax.
- (2) Grade through alcohols to water.
- (3) Stain in the following solution 1 hour: thionin, 0.1 gm.; 5 per cent. solution of phenol in distilled water 100 c.c.
- (4) Grade through alcohols to absolute alcohol.

(5) Differentiate in a saturated solution of orange G in absolute alcohol.

(6) Wash thoroughly in absolute alcohol.

(7) Xylol-alcohol.

(8) Xylol.

(9) Mount in balsam.

The differentiation is accomplished fairly quickly, usually in about  $\frac{1}{2}$  to 1 minute. The progress may be controlled under the microscope, but with a little practice satisfactory differentiation can be carried out by eye observation only. The treatment with orange G is continued until the sections lose their bluish-purple colour and become uniformly yellowish green.

In plant tissues the parasite is stained violet-purple, cellulose walls yellow or green, lignified tissue blue, nuclei pale blue with purple nucleoli and chromosomes in dividing nuclei deep blue on a purple spindle. Nuclei in fungal hyphae are clearly picked out in deep purple. For hand sections the procedure may be shorter.

(1) Sections in water.

(2) Stain in carbol-thionin 5 minutes.

(3) Wash in water.

(4) 95 per cent. alcohol.

(5) Differentiate in the solution of orange G (several minutes).

(6) Wash well in absolute alcohol.

(7) Clear in xylol.

(8) Mount in balsam.

The stain has been found to give good results with such different materials as *Bacterium malvacearum* on *Gossypium*, *B. radicola* in root nodules of legumes, *Plasmodiophora* on *Brassica*, *Synchytrium endobioticum* on *Solanum*, *Peronospora* on *Capsella*, *Phytophthora* and *Sclerotinia* on seedlings, *Botrytis* on *Allium*, and *Puccinia* on *Anemone*. The procedure is so rapid and so easily carried out that the stain combination should prove of value for class purposes.

Apparently any reliable brand of thionin is satisfactory; good results have been obtained with a sample from British Drug Houses and also with the "Soloid" brand tabloids of Messrs Burroughs, Wellcome and Co.

A modification which may prove of value in particular cases is to remove the orange stain by regrading the sections to water after differentiation, and then running up again to xylol. By this means all the orange is removed, leaving the parasite very conspicuous against unstained walls.

REFERENCES.

- (1) VAUGHAN, R. E. (1914). A method for the differential staining of fungus and host cells. *Ann. Mo. Bot. Gard.* 1, 241-2.
- (2) WRIGHT, W. H. and SKORIC, V. (1928). The demonstration of bacteria in plant tissues by means of the Giemsa stain. *Phytopath.* xviii, 803-7.

EXPLANATION OF PLATE VII.

- Fig. 1. *Bacterium malvacearum* in stem of cotton plant. Microtome section of material fixed in Zenker's fluid. Stained thionin and orange G. Photographed with  $\frac{1}{8}$ " achromatic obj.  $\times 10$  Periplanatic ocular. Panchromatic plate. Wratten "M" filter (green)  $\times 530$ .
- Fig. 2. *Peronospora parasitica* in stem of *Capsella*. Hand section of old material in spirit. Stained thionin and orange G. Photographed with  $\frac{3}{8}$ " achromatic obj.  $\times 10$  Periplanatic ocular. Panchromatic plate. Wratten "M" filter (green)  $\times 100$ . Note the differentiation of nuclei in the fungal hyphae.

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Fig. 1.



Fig. 2.

TOUGHTON.—THIONIN AND ORANGE G FOR THE DIFFERENTIAL STAINING OF BACTERIA AND FUNGI IN PLANT TISSUES (pp. 162-164).



